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(54) Title: METHODS OF TREATING CANCER

(57) Abstract: The invention relates to the treatment of cancer using an inhibitor of 5'-methylthioadenosine phosphorylase (MTAP). The invention particularly relates to the treatment of prostate cancer and head and neck cancer.

METHODS OF TREATING CANCER

TECHNICAL FIELD

The present invention relates to methods of treating cancer by administering to a patient in need thereof one or more inhibitors of 5'-methylthioadenosine phosphorylase (MTAP). In particular, the invention relates to methods of treating prostate cancer or head and neck cancer.

BACKGROUND

Certain nucleoside analogues have been identified as potent inhibitors of 5'-methylthioadenosine phosphorylase (MTAP) and 5'-methylthioadenosine nucleosidase (MTAN). These are the subject of US 7,098,334.

Compounds where the location of the nitrogen atom in the sugar ring is varied or where two nitrogen atoms form part of the sugar ring, have also been identified as inhibitors of MTAP and MTAN. These compounds are described in US 10/524,995.

MTAP and MTAN function in the polyamine biosynthesis pathway, in purine salvage in mammals, and in the quorum sensing pathways in bacteria. MTAP catalyses the reversible phosphorolysis of methylthioadenosine (MTA) to adenine and 5-methylthio-α-D-ribose-1-phosphate (MTR-1P). MTAN catalyses the reversible hydrolysis of MTA to adenine and 5-methylthio-α-D-ribose and of S-adenosyl-L-homocysteine (SAH) to adenine and S-ribosyl-homocysteine (SRH). The adenine formed is subsequently recycled and converted into nucleotides. Essentially, the only source of free adenine in the human cell is a result of the action of these enzymes. The MTR-1P is subsequently converted into methionine by successive enzymatic actions.

MTA is a by-product of the reaction involving the transfer of an aminopropyl group from decarboxylated S-adenosylmethionine to putrescine during the formation of spermidine. The reaction is catalyzed by spermidine synthase. Likewise, spermine synthase catalyses the conversion of spermidine to spermine, with concomitant production of MTA as a by-product. The spermidine synthase is very sensitive to product inhibition by accumulation of MTA. Therefore, inhibition of MTAP or MTAN severely limits the polyamine biosynthesis and the salvage pathway for adenine in the cells.

Although MTAP is abundantly expressed in normal cells and tissues, MTAP deficiency due to a genetic deletion has been reported with many malignancies. The loss of MTAP enzyme function in these cells is known to be due to homozygous deletions on chromosome 9 of the closely linked MTAP and *p16/MTS1* tumour suppressor gene. As absence of *p16/MTS1* is probably responsible for the tumour, the lack of MTAP activity is a consequence of the genetic deletion and is not causative for the cancer. However, the absence of MTAP alters the purine metabolism in these cells so that they are mainly dependent on the *de novo* pathway for their supply of purines.

MTA has been shown to induce apoptosis in dividing cancer cells, but to have the opposite, anti-apoptotic effect on dividing normal cells such as hepatocytes (E. Ansorena *et al.*, Hepatology, 2002, 35: 274-280).

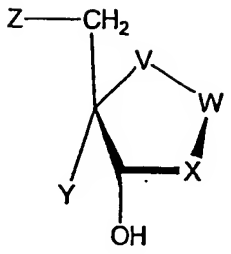
MTAP inhibitors may therefore be used in the treatment of cancer. Such treatments are described in US 7,098,334 and US 10/524,995.

The need for new cancer therapies remains ongoing. For some prevalent cancers the treatment options are still limited. Prostate cancer, for example, is the most commonly diagnosed non-skin cancer in the United States. Current treatment options include radical prostatectomy, radiation therapy, hormonal therapy, and watchful waiting. Although the therapies may offer successful treatment of an individual's condition, the pitfalls are quite unfavorable and lead to a decrease in a man's overall quality of life. Surgery may inevitably result in impotence, sterility, and urinary incontinence. Side effects associated with radiation therapy include damage to the bladder and rectum as well as slow-onset impotence. Hormonal therapy will not cure the cancer and eventually most cancers develop a resistant to this type of therapy. The major risk associated with watchful waiting is that it may result in tumour growth, cancer progression and metastasis. It is therefore desirable that a better treatment option is made available to patients diagnosed with prostate cancer.

It is an object of the invention to provide a method of treating cancer, particularly prostate or head and neck cancer, or at least to provide a useful choice.

STATEMENTS OF INVENTION

In a first aspect, the invention provides a method of treating cancer comprising administering to a patient in need thereof a compound of the formula (I):



(I)

wherein:

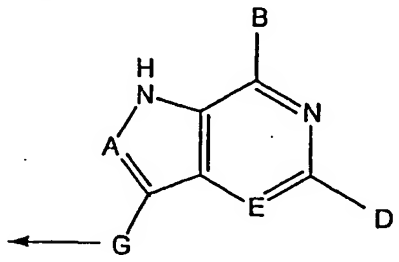
V is selected from CH_2 and NH, and W is selected from CHR^1, NR^1 and NR^2; or V is selected from NR^1 and NR^2, and W is selected from CH_2 and NH;

X is selected from CH_2 and CHOH in the R or S-configuration;

Y is selected from hydrogen, halogen and hydroxy, except where V is selected from NH, NR^1 and NR^2 then Y is hydrogen;

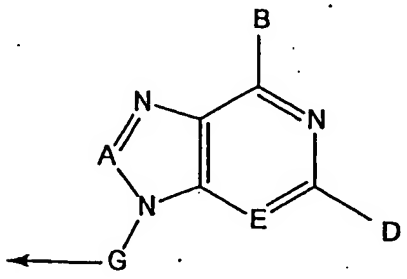
Z is selected from hydrogen, halogen, hydroxy, SQ, OQ and Q, where Q is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, or carboxy;

R^1 is a radical of the formula (II)



(II)

R^2 is a radical of the formula (III)



(III)

A is selected from N, CH and CR^3, where R^3 is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen; or R^3 is hydroxyl, halogen, NH_2, NHR^4, NR^4R^5; or SR^6, where R^4, R^5 and R^6 are alkyl, aralkyl or aryl groups, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen;

B is selected from NH_2 and NHR^7, where R^7 is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen;

D is selected from hydroxy, NH_2, NHR^8, hydrogen, halogen and SCH_3, where R^8 is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen;

E is selected from N and CH;

G is selected from CH_2 and NH, or G is absent, provided that where W is NR^1 or NR^2 and G is NH then V is CH_2, and provided that where V is NR^1 or NR^2 and G is NH then W is CH_2; and provided that where W is CHR^1 then G is absent and V is NH;

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or a prodrug thereof.

Preferably the compound of formula (I) excludes (3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(methylthiomethyl)pyrrolidine.

In preferred embodiments of the invention Z is SQ. In some embodiments Z is not methylthio.

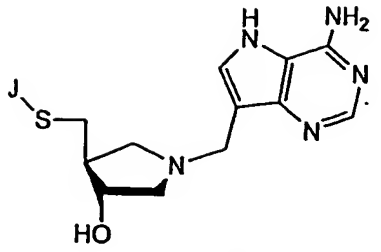
Preferably Q is an alkyl group, optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, and carboxy. It is further preferred that the alkyl group is a C_1-C_6 alkyl group, most preferably a methyl group.

It is also preferred that Q is an aryl group, optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, and carboxy. More preferably the aryl group is a phenyl or benzyl group.

Preferably G is CH_2. It is also preferred that V is CH_2 and W is NR^1. It is further preferred that B is NH_2. It is also preferred that D is H, and it is preferred that A is CH.

Preferably any halogen is chlorine or fluorine.

In preferred embodiments of the invention the compound of formula (I) is a compound of the formula (IV):



(IV)

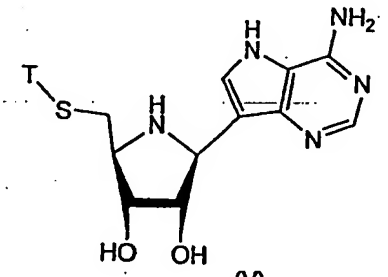
where J is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, and carboxy.

Preferably J is C_1-C_7 alkyl. More preferably J is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexylmethyl, or cycloheptyl.

It is also preferred that J is phenyl, optionally substituted with one or more halogen substituents. More preferably J is phenyl, *p*-chlorophenyl, *p*-fluorophenyl, or *m*-chlorophenyl.

It is also preferred that J is heteroaryl, 4-pyridyl, aralkyl, benzylthio, or $-CH_2CH_2(NH_2)COOH$.

In other preferred embodiments of the invention the compound of the formula (I) is a compound of the formula (V):



(V)

where T is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, and straight- or branched-chain C_1-C_6 alkyl.

Preferably T is C_1-C_6 alkyl, optionally substituted with one or more substituents selected from halogen and hydroxy. More preferably T is methyl, ethyl, 2-fluoroethyl, or 2-hydroxyethyl. Most preferably T is methyl.

It is also preferred that T is aryl, optionally substituted with one or more substituents selected from halogen and straight-chain C_1-C_6 alkyl. More preferably T is phenyl, naphthyl, *p*-tolyl, *m*-tolyl, *p*-chlorophenyl, *m*-chlorophenyl, or *p*-fluorophenyl.

It is also preferred that T is aralkyl. More preferably T is benzyl.

Preferably the compound of formula (I) is:

(3R,4R)-1-[(8-aza-9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(hydroxymethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(2-phenylethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(benzylthiomethyl)pyrrolidine;

(3R,4S)-1-[(8-aza-9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(benzylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(4-chlorophenylthiomethyl)pyrrolidine;

(3R,4R)-1-[(9-deazaadenin-9-yl)methyl]-3-acetoxy-4-(acetoxymethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(*n*-butylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(4-fluorophenylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(*n*-propylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclohexylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(3-chlorophenylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(ethylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(phenylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(4-pyridylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-*n*-propylpyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(homocysteinylmethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(benzyloxymethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(*i*-propylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(methoxymethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclohexylmethylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cycloheptylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclopentylthiomethyl)pyrrolidine;

(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclobutylthiomethyl)pyrrolidine.

It is preferred that the cancer prostate cancer or head and neck cancer.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A shows the survival of mouse prostate cancer cells (RM1) against increasing concentrations of compound (2), either in the presence or absence of MTA.

Figure 1B shows the survival of human prostate cancer cells (PC3) against increasing concentrations of compound (2), either in the presence or absence of MTA.

Figure 2 is a time dependent proliferation curve, showing the effect of compound (2)] and MTA on human prostate cancer cells (PC3).

Figure 3 is a time dependent proliferation curve, showing the effect of compound (2) and MTA on SCC25 cells.

Figure 4 is a time dependent proliferation curve, showing the effect of compound (2) and MTA on FaDu cells.

Figure 5 shows phase contrast photographs of FaDu cells after 5 days of treatment with compound (2) and MTA.

Figure 6 shows a cell cycle and apoptosis analysis of FaDu cells after 6 days of treatment with compound (2) and MTA; (1) untreated results: G1 83.66%, S 8.08%, G2 8.26%, Apoptosis 6.06%; (2) treated with MTA results: G1 79.67%, S 10.42%, G2 9.91%, Apoptosis 6.66%; (3) treated with compound (3) results G1 72.06%, S 17.98%, G29.96%, Apoptosis 7.89%; (4) treated with MTA + compound (3) results G1 8.26%, S 31.25%, G2 60.49%, Apoptosis 29.41%.

Figures 7 to 19 show oral and IP availability of selected compounds that may be used in the methods of the invention, including for compounds (1)-(3) and for ethylthio-DADMe-ImmA, para-chlorophenylthio-DADMe-ImmA, para-fluorophenylthio-DADMe-ImmA, phenylthio-DADMe-ImmA, and phenylthio-ImmA.

Figure 20 shows the effects of compound (2) on FaDu xenografts in NOD-SCID mice.

Figure 21 shows representative tumours from each of the treatment cohorts for the above

NOD-SCID mouse study.

Figure 22 shows MRI images of TRAMP mice (Panels A and B: Control TRAMP (transgenic adenocarcinoma of mouse prostate) mice, Panels E and F: TRAMP mice treated with 1 mM compound (2).

Figure 23 shows that compound (2) and MTA alter polyamine levels and induce cytostasis in PC3 cells (PUT=putrescine, SPD=spermidine, SPN=spermine). PC3 cells were cultured and treated in triplicate as follows: untreated control, 20 μM substrate (MTA) alone, 1 μM compound (2) alone, or a combination of both substrate and inhibitor. Both cells and spent media were harvested at 1, 6, and 12 days for polyamine analysis by HPLC fluorescence.

Figures 24A, 24B and 24C show that compound (2) reduces tumour growth and metastasis in TRAMP mice, but does not alter polyamine levels *in vivo*. C56Bl/6 mice were treated with 100 μM compound (2) via their drinking water and sacrificed at 24, 48 hours, and 7 days. Livers were immediately removed for polyamine analysis. TRAMP mice were treated approximately 6-8 months with 100 μM compound (2) via their drinking water and control sacrificed. Livers were removed for polyamine analysis.

Figures 25A and 25B show Cal27 cells grown for 8 days as control (untreated), in the presence of 20 μM MTA, 1 μM compound (2) alone or in combination (1 μM compound (2) + 20 μM MTA).

Figure 26 shows mouse lung cancer cells in culture responding to compound (1) in the presence of 20 μM MTA and not responding in the absence of MTA.

DETAILED DESCRIPTION

Definitions

The term "alkyl" is intended to include straight- and branched-chain alkyl groups, as well as cycloalkyl groups. The same terminology applies to the non-aromatic moiety of an aralkyl radical. Examples of alkyl groups include: methyl group, ethyl group, *n*-propyl group, *iso*-propyl group, *n*-butyl group, *iso*-butyl group, *sec*-butyl group, *t*-butyl group, *n*-pentyl group, 1,1-dimethylpropyl group, 1,2-dimethylpropyl group, 2,2-dimethylpropyl group, 1-ethylpropyl group, 2-ethylpropyl group, *n*-hexyl group and 1-methyl-2-ethylpropyl group.

The term "aryl" means an aromatic radical having 6 to 18 carbon atoms and includes heteroaromatic radicals. Examples include monocyclic groups, as well as fused groups such

as bicyclic groups and tricyclic groups. Some examples include phenyl group, indenyl group, 1-naphthyl group, 2-naphthyl group, azulenyl group, heptalenyl group, biphenyl group, indacenyl group, acenaphthyl group, fluorenyl group, phenalenyl group, phenanthrenyl group, anthracenyl group, cyclopentacyclooctenyl group, and benzocyclooctenyl group, pyridyl group, pyrrolyl group, pyridazinyl group, pyrimidinyl group, pyrazinyl group, triazolyl group, tetrazolyl group, benzotriazolyl group, pyrazolyl group, imidazolyl group, benzimidazolyl group, indolyl group, isoindolyl group, indolizinyl group, purinyl group, indazolyl group, furyl group, pyranyl group, benzofuryl group, isobenzofuryl group, thienyl group, thiazolyl group, isothiazolyl group, benzothiazolyl group, oxazolyl group, and isoxazolyl group.

The term "halogen" includes fluorine, chlorine, bromine and iodine.

The compounds are useful for the treatment of certain diseases and disorders in humans and other animals. Thus, the term "patient" as used herein includes both human and other animal patients.

The term "prodrug" as used herein means a pharmacologically acceptable derivative of the compound of formula (I), (IV) or (V), such that an *in vivo* biotransformation of the derivative gives the compound as defined in formula (I), (IV) or (V). Prodrugs of compounds of formulae (I), (IV) or (V) may be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved *in vivo* to give the parent compound.

Prodrugs include compounds of formulae (I), (IV) or (V), tautomers thereof and/or pharmaceutically acceptable salts thereof, which include an ester functionality, or an ether functionality. It will be clear to the skilled person that the compounds of formulae (I), (IV) or (V) may be converted to corresponding ester or ether prodrugs using known chemical transformations. Suitable prodrugs include those where the hydroxyl groups of the compounds of formula (I), (IV) or (V) are esterified to give, for example, a primary hydroxyl group ester of propanoic or butyric acid. Other suitable prodrugs are alkycarbonyoxymethyl ether derivatives on the hydroxyl groups of the compounds of formula (I), (IV) or (V) to give, for example, a primary hydroxyl group ether with a pivaloyloxymethyl or a propanoyloxymethyl group.

The term "pharmaceutically acceptable salts" is intended to apply to non-toxic salts derived from inorganic or organic acids, including, for example, the following acid salts: acetate,

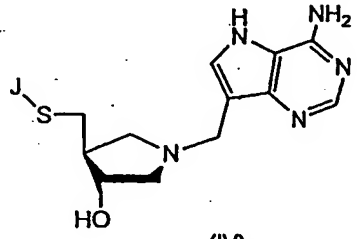
adipate, alginate, aspartate, benzoate, benzenesulfonate, bisulfate, butyrate, citrate, camphorate, camphorsulfonate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptanoate, glycerophosphate, glycolate, hemisulfate, heptanoate, hexanoate, hydrochloride, hydrobromide, hydroiodide, 2-hydroxyethanesulfonate, lactate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oxalate, palmoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, *p*-toluenesulfonate, salicylate, succinate, sulfate, tartrate, thiocyanate, and undecanoate.

Discussion of Cancer Treatment

The present invention relates to methods of treating cancer by administering to a patient in need thereof one or more inhibitors of 5'-methylthioadenosine phosphorylase (MTAP). In particular, the invention relates to methods of treating certain cancers, such as prostate cancer or head and neck cancer.

Suitable MTAP inhibitors which may be employed in the method of the present invention and the methods for preparing these inhibitors are described in US 7,098;334 and US 10/524,995.

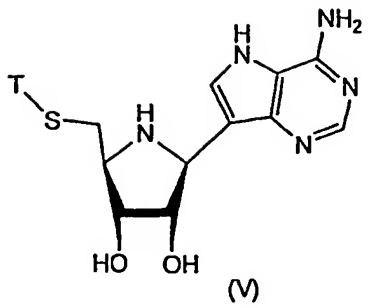
Certain MTAP inhibitor compounds are surprisingly effective for treating prostate and head and neck cancers. These are compounds of general formula (IV).



(IV)

This sub-dass of MTAP inhibitors incorporates an adenine-like base moiety and a pyrrolidine moiety having an alkyl- aryl- or aralkylthiomethyl group at the 4-position.

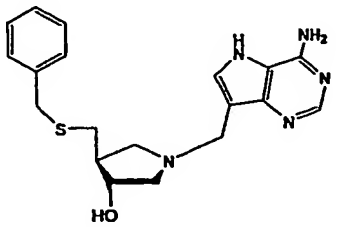
Other MTAP inhibitor compounds are also surprisingly effective for treating prostate and head and neck cancers. These are compounds of general formula (V).



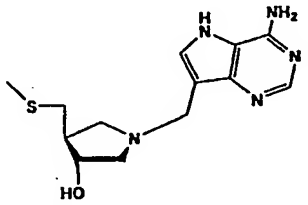
(V)

This sub-class of MTAP inhibitors also incorporates the adenine-like base moiety but has an iminoribitol moiety with an alkyl- aryl- or aralkylthiomethyl group at the 5'-position.

Examples of the first sub-class of inhibitors include compounds (1) and (2).



Compound (1)
BT-DADMe-ImmA



Compound (2)
MT-DADMe-ImmA

The Examples below show that compounds (1) and (2) are effective both *in vitro* and *in vivo* against a variety of cell lines (PC3, RM1, SCC25 and FaDu). These compounds are therefore particularly useful in the treatment of prostate and head and neck cancers.

The MTAP inhibitor compounds inhibit cell growth *in vitro* of the prostate cancer cell lines PC3 and RM1 and the head and neck cancer cell lines SCC25 and FaDu. An enhanced cell-killing effect is seen *in vitro* with combined administration of the MTAP inhibitor compound plus MTA. Examples of this effect are shown in Figures 1 to 6.

Furthermore, the inhibitor compounds, when co-administered with MTA, exhibit a cytostatic effect on PC3 cells *in vitro*.

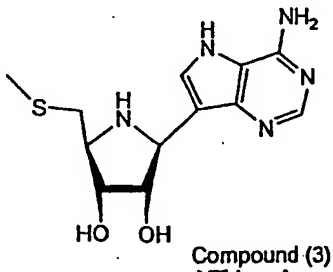
In order to determine whether the inhibition is selective for malignant cells, normal human fibroblast cells (GM02037) were also treated with compound (2) and MTA for 3 weeks. No cytotoxicity was observed. Compound (2) is therefore cytotoxic for human HNSCC (human head and neck squamous cell carcinoma) cells at doses that exhibit minimal toxicity for normal cells. This selectivity is a further indication that the MTAP inhibitors described above

are useful agents for the treatment of head and neck cancer.

The present *in vivo* studies further demonstrate the surprising efficacy of the compounds. In a NOD-SCID mouse model, compound (2) significantly delays the growth of established FaDu xenografts. The effect is seen either with or without co-administration of the inhibitor compound with MTA.

In addition, prostate cancer progression in the TRAMP mouse model is inhibited in mice treated with compound (2), either alone or in combination with MTA.

An example of the second sub-class of inhibitors is compound (3).



Compound (3)
MT-ImmA

This compound also inhibits prostate cancer progression in the TRAMP mouse model, when administered either alone or in combination with MTA.

For the above *in vivo* models, the inhibitor compounds exhibit activity when administered with exogenous MTA and when administered alone. There is not a significant enhancement observed when the inhibitors are administered together with MTA. However, the *in vitro* results clearly demonstrate a surprising enhancement in activity when the inhibitors are administered in conjunction with MTA. Thus, the combined administration method provides a potential alternative treatment method for patients suffering from cancer, where the administration of an MTAP inhibitor is indicated.

The MTAP inhibitor compounds of formulae (I), (IV) and (V) (in particular the compounds of formulae (IV) and (V)) provide an effective alternative treatment option for cancer sufferers, especially for patients diagnosed with prostate and head and neck cancers.

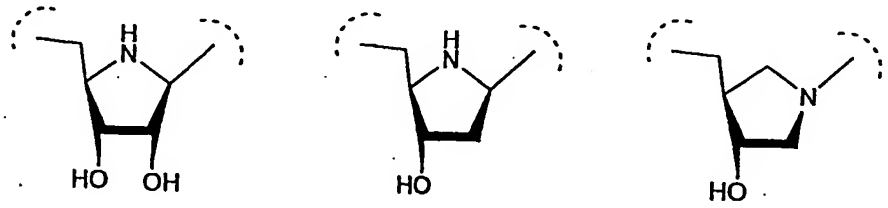
General Aspects

The MTAP inhibitor compounds are useful in both free base form and in the form of salts.

It will be appreciated that the representation of a compound of formula (I) where B and/or D is a hydroxy group, is of the enol-type tautomeric form of a corresponding amide, and this will largely exist in the amide form. The use of the enol-type tautomeric representation is simply to allow fewer structural formulae to represent the compounds of the invention.

Similarly, it will be appreciated that the representation of a compound of formula (I), where B and/or D is a thiol group, is of the thioenol-type tautomeric form of a corresponding thioamide, and this will largely exist in the thioamide form. The use of the thioenol-type tautomeric representation is simply to allow fewer structural formulae to represent the compounds of the invention.

It will also be appreciated that the compounds depicted with bold solid lines are representations of the D-ribo or 2'-deoxy-D-erythro- stereochemical arrangement of substituents on the pyrrolidine ring, such as shown here.



Formulations and Modes of Administration

Figures 7, 9, 10, 12, 13, 15 and 16-19 show that the MTAP inhibitor compounds used in the methods of the present invention are orally available, and may therefore be formulated for oral administration. The compounds may also be administered by other routes. For example, the MTAP inhibitors may be administered to a patient orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally or via an implanted reservoir. The amount of compound to be administered will vary widely according to the nature of the patient and the nature and extent of the disorder to be treated. Typically the dosage for an adult human will be in the range less than 1 to 1000 milligrams, preferably 0.1 to 100 milligrams. The specific dosage required for any particular patient will depend upon a variety of factors, including the patient's age, body weight, general health, sex, etc.

For oral administration the active compounds can be formulated into solid or liquid preparations, for example tablets, capsules, powders, solutions, suspensions and dispersions. Such preparations are well known in the art as are other oral dosage regimes not listed here. In the tablet form the compounds may be tableted with conventional tablet bases such as lactose, sucrose and corn starch, together with a binder, a disintegration

agent and a lubricant. The binder may be, for example, corn starch or gelatin, the disintegrating agent may be potato starch or alginic acid, and the lubricant may be magnesium stearate. For oral administration in the form of capsules, diluents such as lactose and dried cornstarch may be employed. Other components such as colourings, sweeteners or flavourings may be added.

When aqueous suspensions are required for oral use, the active ingredient may be combined with carriers such as water and ethanol, and emulsifying agents, suspending agents and/or surfactants may be used. Colourings, sweeteners or flavourings may also be added.

The compounds may also be administered by injection in a physiologically acceptable diluent such as water or saline. The diluent may comprise one or more other ingredients such as ethanol, propylene glycol, an oil, or a pharmaceutically acceptable surfactant.

The compounds may also be administered topically. Carriers for topical administration of the compounds include mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene, polyoxypropylene compound, emulsifying wax and water. The compounds may be present as ingredients in lotions or creams, for topical administration to skin or mucous membranes. Such creams may contain the active compounds suspended or dissolved in one or more pharmaceutically acceptable carriers. Suitable carriers include mineral oil, sorbitan monostearate, polysorbate 60, cetyl ester wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

The compounds may further be administered by means of sustained release systems. For example, they may be incorporated into a slowly dissolving tablet or capsule.

Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences (Mack Publishing Company).

EXAMPLES

Inhibitor Compounds Inhibitors of MTAP were synthesized as described earlier (Singh, V., Shi, W., Evans, G. B., Tyler, P. C., Furneaux, R H, Almo, S C, and Schramm, V L (2004) *Biochemistry* 43, 9-18; Evans G B, Furneaux R H, Lenz D H, *et al.*, *J Med Chem* 2005:48, 4679-89). Solutions were standardized by the UV absorbance of the 9-deazaadenine ring. Sterile solutions of inhibitors were prepared by filtration.

Protocol for Clonogenic Survival Assay of Cancer Cells

1. 60% confluent plates of experimental cell line was taken and subjected to trypsinization
2. Single cell suspension of the experimental cell line was made in the regular growth medium and number of cells per mililitre of suspension counted
3. A fixed low number of cells were plated out in a volume of 3ml of growth medium in each well of 6 well culture dishes and incubated overnight at 37°C in a CO_2 incubator
4. Measured volumes of the inhibitor and substrate solutions in sterile deionised cell culture water was added to each well of the 6 well plates. Typically each concentration of inhibitor and/or substrate was added in triplicate wells to calculate error bars. Final concentrations were calculated based on a total volume of 3ml of culture medium such that dilution factor did not exceed 1% of final volume.
5. Treated cell culture plates were incubated at 37°C in a CO_2 incubator for a period of 7 days
6. At the end of the period of incubation growth medium was removed from each well, attached cells were washed once with PBS and fixed by addition of 100% Formalin solution to each well and keeping at room temperature for ~1 hour.
7. At the end of 1 hour, formalin was removed from the wells and ~150µL of Crystal Violet staining solution was added to each well and let stand at room temperature for 30 min.
8. After staining is complete, wells were flushed with running tap water to remove traces of residual stain and dried by inverting over paper towels.
9. Number of crystal violet stained colonies in each well containing more than 60 cells per colony was counted.
10. Assuming each colony originated from a single surviving cell post-treatment and taking the number of colonies in the untreated control well as 1, the fraction of surviving cells in each well was calculated and plotted in a graph.

Example 1: Clonogenic Assays (Figures 1A and 1B) for Compound (2)

PC3 cells were grown in equal (1:1) portions of Dulbecco's modified Eagle's medium and F12 containing 10% fetal bovine serum, 10 U/mL penicillin-G and 10 µg/mL streptomycin in monolayers to near confluency at 37 °C. Cells were lysed in 50 mM sodium phosphate pH 7.5, 10 mM KCl and 0.5% Triton X-100.

Example 2: Effect of Compound 2 and MTA on PC3 cells (Figure 2)

PC3 cells were maintained in MEM Eagle's media supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 µg/mL streptomycin, 0.1 mM non essential amino acids and 1 mM sodium pyruvate. Cell survival was evaluated using the WST-1 assay (Kicska G

A, long Li, Horig H, *et al. Proc Natl Acad Sci USA* 2001;98:4593-98). Cells were seeded onto 96 well plates at a density of 10^4 cells per well, with either no additions, 1 μM compound (2), 20 μM MTA or 1 μM compound (2) + 20 μM MTA. IC_{50} was determined following the manufacturer's protocol (Roche Applied Science, IN). Cells were grown and measured in triplicate or quadruplicate and the error bars show the mean ± SD of the multiple samples.

Example 3: Effect of Compound 2 and MTA on SCC25 cells (Figure 3)

SCC25 cells were maintained in MEM Eagle's media supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/mL streptomycin, 0.1 mM non essential amino acids and 1 mM sodium pyruvate. Cell survival was evaluated using the WST-1 assay (Kicska G A, long Li, Horig H, *et al. Proc Natl Acad Sci USA* 2001;98:4593-98). Cells were seeded onto 96 well plates at a density of 10^4 cells per well, with either no additions, 1 μM MT-compound (2), 20 μM MTA or 1 μM compound (2) + 20 μM MTA. IC_{50} was determined following the manufacturer's protocol (Roche Applied Science, IN). Cells were grown and measured in triplicate or quadruplicate and the error bars show the mean ± SD of the multiple samples.

Example 4: Effect of MT-DADMe-ImmA (Compound (2)) and MTA on FaDu cells (Figure 4)

FaDu cells were maintained in MEM Eagle's media supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/mL streptomycin, 0.1 mM non essential amino acids and 1 mM sodium pyruvate. Cell survival was evaluated using the WST-1 assay (Kicska G A, long Li, Horig H, *et al. Proc Natl Acad Sci USA* 2001;98:4593-98). Cells were seeded onto 96 well plates at a density of 10^4 cells per well, with either no additions, 1 μM compound (2), 20 μM MTA or 1 μM compound (2) + 20 μM MTA. IC_{50} was determined following the manufacturer's protocol (Roche Applied Science, IN). Cells were grown and measured in triplicate or quadruplicate and the error bars show the mean ± SD of the multiple samples.

Example 5: Phase Contrast Microscopy of FaDu Cells (Figure 5)

FaDu cells were subjected to six days in culture using the same conditions described as for Example 4.

Example 6: Cell Cycle and Apoptosis Analysis of FaDu cells (Figure 6)

FaDu cells were subjected to six days in culture using the same conditions described as for Example 4, before staining with propidium bromide and FACS cell sorting analysis.

Example 7: Oral Availability (Compound (2))

Two groups of 3 C57BL6 mice received a single oral dose of compound (2) dissolved in sterile, deionized water, pippeted onto a crumb of food. Treated food was fed to each mouse individually under close observation at time zero. Two different single doses of inhibitor were administered: 50 µg and 100 µg. Mice were individually fed and closely observed for consumption of food. At specific time points, 4 µL blood samples were collected from the tail vein. The blood was mixed with 4µL 0.6% Triton X-100 in PBS and stored at -80°C until time of analysis. The amount of adenine produced was measured by the following MTAP activity assay: Cells were harvested, washed three times with PBS and lysed with RIPA buffer. The reaction mixture for MTAP activity assays contained the following: ~ 75 µg protein from cell lysates, 50 mM HEPES pH 7.4, 50 µM MTA, and 20,000 dpm [2,8-3H]MTA. Labeled MTA was synthesized from [2,8-3H]S-adenosylmethionine by a known method. Products of the MTAP reaction were resolved using TLC silica plates with 1 M ammonium acetate, pH 7.55, and 5% isopropanol. Adenine spots were excised and counted for label incorporation.

Example 8: Oral and IP Availability for Selected Compounds (Figures 7 to 19)

Oral dosing was performed in essentially the same manner as for Example 7. For IP availability, 100 µg of the inhibitor was dissolved in around 200µl of sterile deionised water and taken up in a 1ml syringe attached to a 26G needle and injected intraperitonially in the mouse at 0min time point. Blood (4µl) was collected from the tail of the mouse at specific time points, mixed with 4µl of 0.6% TritonX-100 solution in PBS and stored at -80°C until ready for enzyme assay. Blood (4µl) was collected from each mouse prior to injection which served as 0min control time point. Each experiment was repeated three times with three different mice to get standard error bars.

Example 9: FaDu Xenograft Studies (Figures 20 and 21)

NOD-SCID mice (6-8 weeks old) were obtained from Jackson Laboratory (Bar Harbor, Maine). FaDu cells (10^6) were inoculated into the dorsum of the hind foot. After 5 days, mice were treated with 9 mg/kg or 21 mg/kg body weight of compound (2) in drinking water or by daily i.p. injections of 5 mg/kg body weight of compound (2). After inoculation mice were assigned to treatment or control groups (n = 5). Tumor volume (V) was determined from: V = (4/3)*(22/7)*1/8*(length*width*height). Differences between treatment cohorts were determined using the Student's t test. Mice were weighed every 4-5 days, monitored for hair loss, loss of appetite, vomiting and diarrhoea. Total and differential blood and bone marrow analyses were performed after treatment with compound (2).

Example 10: MRI Studies (Figure 22)

MRI experiments were performed using a 9.4T 21 cm bore horizontal bore magnet (Magnex Scientific) Varian INOVA MRI system (Fremont, CA) equipped with a 28 mm inner diameter quadrature birdcage coil. Mice were anesthetized with isoflurane inhalation anesthesia (1-1.5% in 100% O_2 administered via a nose cone) and positioned in the MRI coil. Body temperature was maintained (37-38°C) using a homeothermic warming system. After acquiring scout images, multi-slice spin-echo imaging with an echo time of 18 ms and a repetition time of 400ms ms was performed. A 40 mm field of view with a 256 x 256 matrix size was used. Nine to 15 slices along the transverse, sagittal, and coronal planes were acquired in each multi-slice experiment with a slice thickness of 1 mm and the gap between slices of 0.5 mm. MRI data were processed off-line with MATLAB-based MRI analysis software.

Example 11: Quantitation of Polyamines in Cells, Spent Media and Tissue Samples (Figure 23)

Spent media and perchloric acid extracts of both PC3 cells and tissue samples were subjected to purification via cation exchange chromatography and dansyl-derivatized with minor changes. Disposable 10 ml BIO-RAD columns were centrifuged at 4,000 rpm for 3 minutes. Sodium carbonate used for derivatization was adjusted to pH 9.3 and the concentration of dansyl-chloride was adjusted to 100 mM. Dansyl-polyamines were quantitated by a Waters HPLC/ Fluorescence system. A Phenomenex Luna 5 µ C18 column was used with a mobile phase of 30% acetonitrile in a 50 mM ammonium acetate buffer at pH 6.8 (eluent A) and 100% acetonitrile (eluent B). Fluorescence detection was monitored by excitation at 338 nm and emission at 500 nm.

Example 12: Treatment of TRAMP Mice (Table 1, Figure 22)

Short-Term: Mice were treated with sterile solutions of 100 µM compound (2) (pH ~6.4). Water bottles were autoclaved prior to filling with sterile inhibitor solutions. Mice were sacrificed at 1, 2, and 7 days, with three mice in each group, with the control group sacrificed after 7 days. Livers were immediately removed upon sacrifice for polyamine analysis, conducted as described above.

Long-Term: Sterile solutions of 100 µM compound (2) (pH ~6.4). Water bottles were autoclaved prior to filling with sterile inhibitor solutions. Water consumption was monitored every other day, with fresh inhibitor solution being administered to prevent bacterial growth. Mice were control-sacrificed and tissues (genitourinary system, liver, lungs) were collected for histology and polyamine analysis. Mass and dimensions of excised genitourinary system

tumours were recorded. Sections of small intestine were also removed for toxicity analysis via H&E staining.

Example 13: Mouse 3LL Cell Studies for Compound (1) (Figure 26)

Growth of 3LL and RM1 cells was in Dulbecco's modified Eagle's medium containing serum and antibiotics with 5 mM sodium pyruvate and 0.25 mM non essential amino acid mixture (Gibco). Compound (1) was added as a sterile solution and MTA was absent or present at 20 μM.

Discussion of the Examples

Figure 1A shows the effect of the addition of compound (2) to cultured mouse prostate cancer cells (RM1). Figure 1B shows the effect of the addition of compound (2) to cultured human prostate cancer cells (PC3). Compound (2) was added either alone or in the presence of 20 μM MTA. Figures 2, 3 and 4 show the effects of MTA alone, compound (2) alone, and MTA with compound (2) in time dependent cell proliferation experiments (PC3 cells, SCC25 cells and FaDu cells). The combination of compound (2) and MTA reduces cell proliferation. These data demonstrate that the compounds which are used in the methods of the present invention inhibit cell growth *in vitro*, when administered in combination with MTA.

Figure 5 further demonstrates, showing phase contrast photographs of FaDu cells after 5 days of treatment with compound (2)/compound (2) + MTA, that the inhibitor compound + MTA is effective in inhibiting cell growth.

Thus, administration of MTA in circumstances where its degradation by MTAP is inhibited by an MTAP inhibitor leads to greater circulatory and tissue levels of MTA and consequently an enhanced effect in the treatment of cancer.

Figure 6 shows that compound (2) in combination with MTA is also effective for stopping cell cycling (for FaDu cells) such that the cells become apoptotic.

Figures 7 to 19 show oral and IP availability of selected compounds, including compounds (1)-(3) and ethylthio-DADMe-ImmA, para-chlorophenylthio-DADMe-ImmA, para-fluorophenylthio-DADMe-ImmA, phenylthio-DADMe-ImmA, and phenylthio-ImmA.

Figures 20 and 21 show the results of *in vivo* studies. The time-dependent growth of FaDu tumors in immunodeficient mice was suppressed by oral or intraperitoneal treatment with compound (2) (Figure 20). Tumors were established in mice for 5 days prior to oral or

interperitoneal treatments with compound (2). Tumor growth in animals treated with compound (2) was dose responsive and was significantly slower than in controls ($p < 0.06$). Representative tumors from the treatment cohorts are shown at 28 days after therapy began (Figure 21). No significant differences in animal weight or in total and differential blood counts were seen between treatment and control groups after this treatment. Thus, compound (2) administration suppresses FaDu growth *in vivo* with low cytotoxicity. Subsequent to the 28 day compound (2) therapy, treatment was removed for a subsequent period of 28 days. There was no regrowth of tumor in those mice receiving the two highest doses of compound (2).

Another head and neck cancer cell line, Cal27 was also found to be susceptible to compound (2) and MTA. After 8 days of treatment, the number of viable Cal27 cells decreased as a result of G_2/M arrest and apoptosis when compared to controls (Figures 25A and 25B).

Longitudinal MRI provides a noninvasive means of monitoring prostate tumour growth in mice (Gupta S, Hastak K, Ahmad N, Lewin J S, Mukhtar H *Proc Natl Acad Sci USA* 2001 Aug 28;98(18):10350-5; Eng M H, Charles L G, Ross B D, Chrisp C E, Pienta K J, Greenberg N M, Hsu C X, Sanda M G *Urology* 1999 Dec:54(6):1112-9; Song S K, Qu Z, Garabedian E M, Gordon J I, Milbrandt J, Ackerman J J *Cancer Res.* 2002 Mar 1:62(5):1555-8.).

MRI was used to evaluate prostate tumour growth and progression longitudinally in TRAMP mice (either untreated or treated with a compound that may be used according the methods of the invention). Mice were imaged approximately monthly from 12-33 weeks of age. Representative MRI images comparing untreated control TRAMP and treated TRAMP mice at approximately 30 weeks of age are shown in Figure 22.

Panels A and B show results from control mice. Panel A shows a coronal section through of a 30 week old TRAMP mouse with a large tumour (bright tissue) that weighed 8.76 g upon dissection at 34 weeks of age. The inset shows a more posterior coronal section. The bright tumour is smaller in this section but metastasis to the liver is observed (white arrow). Panel B shows a coronal section through the prostate region of a 30 week old TRAMP mouse. The seminal vesicles (SV) are enlarged. A large tumour (weighing 4.89 g upon dissection at 36 weeks of age) that spanned from the kidney to bladder (BL) is visible in the transverse section shown in the inset (white arrow).

Panels E and F show results for mice treated with 1 mM compound (2). Panel E shows a coronal section through the prostate region of a 30 week old treated TRAMP mouse. The tumour, weighing 0.41 g upon dissection at 34 weeks of age, was not observed during the imaging session. Panel F shows a similar section through a 30 week old treated TRAMP mouse that exhibited a 0.64 g tumour upon dissection at 39 weeks of age. The tumour is indicated by the white arrow in the MRI image shown in this panel.

Untreated TRAMP mice therefore demonstrate primary prostate tumour growth. However, prostate cancer progression in the TRAMP mouse is inhibited in mice treated with compound (2), either alone or in combination with MTA.

Figure 23 shows that compound (2) and MTA, administered together, alter polyamine levels and induce cytostasis in PC3 cells. Combination treatment of PC3 cells with compound (2) and MTA for 1 day resulted in a significant 6-fold increase in intracellular PUT levels ($3.03 \times 10^{-3} \pm 2.86 \times 10^{-2}$, combination treated cells vs. $5.04 \times 10^{-2} \pm 1.08 \times 10^{-2}$, control, p= 0.001, pmoles PUT/mg protein), a 2-fold increase in spent media PUT levels [$1.19 \times 10^{-3} \pm 2.04 \times 10^{-1}$, combination treated media vs. $5.85 \times 10^{-2} \pm 5.09 \times 10^{-0}$, control media, p= 0.0001, pmoles PUT/mL spent media, as well as roughly a 2.5-fold increase in intracellular SPD levels ($7.19 \times 10^{-3} \pm 4.38 \times 10^{-2}$, combination treated cells vs. $3.05 \times 10^{-3} \pm 6.3 \times 10^{-2}$, control, p=0.001 pmoles SPD/mg protein). SPN levels in combination treated spent media also slightly decreased (p=0.02). After 6 days of treatment, cellular SPN levels were decreased roughly 0.5-fold ($4.0 \times 10^{-3} \pm 7.38 \times 10^{-2}$, combination treated cells vs. $6.87 \times 10^{-3} \pm 9.68 \times 10^{-2}$, control, p= 0.005, pmoles SPN/mg protein), with both PUT and SPD elevated (p= 0.02 and p= 0.01, respectively in comparison to controls). Most significantly, levels of PUT in spent media were almost double that of the control ($2.41 \times 10^{-3} \pm 7.35 \times 10^{-1}$, combination treated spent media vs. $1.31 \times 10^{-3} \pm 0.0$, control, p=0.0007, pmoles PUT/mL spent media). By day 12, a significant increase in cellular SPD levels were observed ($9.05 \times 10^{-3} \pm 1.09 \times 10^{-3}$, combination treated cells vs. $3.93 \times 10^{-3} \pm 8.4 \times 10^{-1}$, control, p=0.007, pmoles SPD/mg protein), with a corresponding decrease in levels of spent media PUT levels (1.65 x 10-3 $\pm$ 2.27×10^{-2}, combination treated spent media vs. $2.12 \times 10^{-3} \pm 9.34 \times 10^{-1}$, control media, pmoles PUT/mL spent media, p=0.013). Intracellular PUT levels in combination treated cells were still significantly higher than controls (p=0.005).

Treatment of PC3 cells with compound (2) resulted in numerous significant alterations in both intracellular and spent media polyamine levels. After 24 hours of treatment, the increase observed in cellular SPD levels as well as putrescine (PUT) cellular and spent media polyamine levels correlated with the effects expected with MTAP inhibition. MTA

accumulated in the cells, began feedback inhibition of SPN synthase, resulting in accumulations of SPD and PUT, with PUT being significantly excreted into the media, and a slight decrease of SPN in the media. By day 6, cellular SPN levels were significantly reduced in combination treated cells, while maintaining the characteristic elevations in levels of PUT and SPD. Treatment of cells for 12 days showed a significant increase in cellular SPD levels and a slight decrease in spent media PUT levels, pointing to the fact that a compensatory pathway had been activated to make up for the block in MTAP. PUT may have been being taken up from the media for SPD synthesis. After combination treatment for approximately 2 weeks, PC3 cells displayed a cytostatic effect, as determined by the clonogenic assay. Initially, it was believed that MTAP inhibition would lead to MTA accumulation, causing feedback inhibition of polyamine biosynthesis, resulting in decreases in cellular proliferation. Although a halt in cellular proliferation was observed, this is clearly not due simply to polyamine depletion.

Figures 24A-C show that compound (2) reduces tumour growth and metastasis in TRAMP mice, but does not alter polyamine levels *in vivo*. Polyamine levels of mice livers were not significantly altered during short-term treatment (Figure 24A). After extended treatment with compound (2) inhibitor solutions, no significant alterations in either TRAMP liver or GUS polyamine levels were detected (Figures 24B and 24C).

Mass (Table 1) and dimensions of excised genitourinary system tumors were recorded for all members of the treatment groups. Sections of small intestine were also removed for toxicity analysis via H&E staining. Histology of TRAMP mice revealed all animals showed extensive prostate intraepithelial neoplasia involving most prostate acini. However, the size and incidence of preinvasive tumors, as well as the incidence of invasive cancer and metastasis were all decreased in treated TRAMP mice (Table 1). No alterations, inflammations, or irregularities were observed in the intestinal crypts, neither were any hair loss or general GI problems noted, indicating a lack of drug toxicity.

Table 1: Summary of results for TRAMP mice treated with compound (2)

Experimental Condition	# Animals (n)	Tumor Size (g)	Weeks treated	Metastatic Cancer
Control	16	4.0 ± 2.8	32 ± 5	44%
100 μM compound (2)	12	1.7 ± 0.8	29 ± 7	8%

Figure 26 shows mouse lung cancer cells in culture responding to compound (1) in the presence of 20 μM MTA and not responding in the absence of MTA. This establishes that the effect of the inhibitor is on MTAP and that cancer cell lines are susceptible to this treatment.

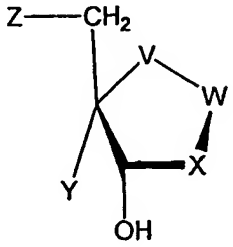
Although the invention has been described by way of example, it should be appreciated the variations or modifications may be made without departing from the scope of the invention. Furthermore, when known equivalents exist to specific features, such equivalents are incorporated as if specifically referred to in the specification.

INDUSTRIAL APPLICABILITY

Compounds that are inhibitors of MTAP inhibitors have potential for treating cancer, particularly prostate cancer and head and neck cancer.

CLAIMS

1. A method of treating cancer comprising administering to a patient in need thereof a compound of the formula (I):



(I)

wherein:

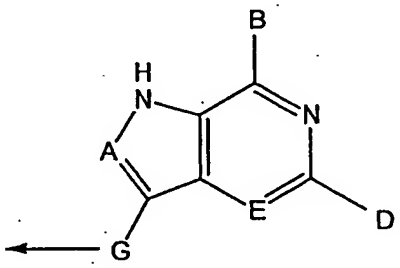
V is selected from CH_2 and NH, and W is selected from CHR^1, NR^1 and NR^2; or V is selected from NR^1 and NR^2, and W is selected from CH_2 and NH;

X is selected from CH_2 and CHOH in the R or S-configuration;

Y is selected from hydrogen, halogen and hydroxy, except where V is selected from NH, NR^1 and NR^2 then Y is hydrogen;

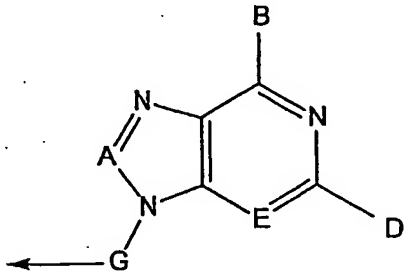
Z is selected from hydrogen, halogen, hydroxy, SQ, OQ and Q, where Q is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, or carboxy;

R^1 is a radical of the formula (II)



(II)

R^2 is a radical of the formula (III)



(III)

A is selected from N, CH and CR^3, where R^3 is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy and

halogen; or R^3 is hydroxyl, halogen, NH_2, NHR^4, NR^4R^5; or SR^6, where R^4, R^5 and R^6 are alkyl, aralkyl or aryl groups, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen;

B is selected from NH_2 and NHR^7, where R^7 is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen;

D is selected from hydroxy, NH_2, NHR^8, hydrogen, halogen and SCH_3, where R^8 is alkyl, aralkyl or aryl, each of which is optionally substituted with one or more substituents selected from hydroxy and halogen;

E is selected from N and CH;

G is selected from CH_2 and NH, or G is absent, provided that where W is NR^1 or NR^2 and G is NH then V is CH_2, and provided that where V is NR^1 or NR^2 and G is NH then W is CH_2; and provided that where W is CHR^1 then G is absent and V is NH;

or a tautomer thereof, or a pharmaceutically acceptable salt thereof, or a prodrug thereof.

2. A method as claimed in claim 1 where the compound of formula (I) excludes (3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(methylthiomethyl)pyrrolidine.

3. A method as claimed in claim 1 or claim 2 where Z is SQ.

4. A method as claimed in claim 3 where Z is not methylthio.

5. A method as claimed in claim 3 where Q is an alkyl group, optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, and carboxy.

6. A method as claimed in claim 5 where the alkyl group is a C_1-C_6 alkyl group.

7. A method as claimed in claim 6 where the C_1-C_6 alkyl group is a methyl group.

8. A method as claimed in claim 3 where Q is an aryl group, optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, and carboxy.

9. A method as claimed in claim 8 where the aryl group is a phenyl or benzyl group.

10. A method as claimed in any one of claims 1 to 9 where G is CH_2.

11. A method as claimed in claim 10 where V is CH_2 and W is NR^1.

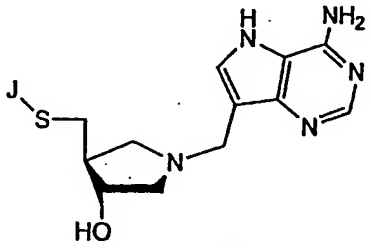
12. A method as claimed in any one of claims 1 to 11 where B is NH_2.

13. A method as claimed in any one of claims 1 to 12 where D is H.

14. A method as claimed in any one of claims 1 to 13 where A is CH.

15. A method as claimed in any one of claims 1 to 14 where any halogen is chlorine or fluorine.

16. A method as claimed in claim 1 where the compound of the formula (I) is a compound of the formula (IV):



(IV)

where J is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, and carboxy;
or a pharmaceutically acceptable salt thereof, or a prodrug thereof.

17. A method as claimed in claim 16 where J is C_1-C_7 alkyl.

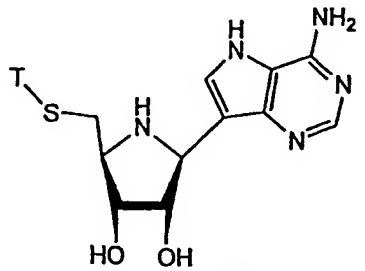
18. A method as claimed in claim 17 where J is methyl, ethyl, *n*-propyl, *i*-propyl, *n*-butyl, cyclobutyl, cyclopentyl, cyclohexyl, cyclohexylmethyl, or cycloheptyl.

19. A method as claimed in claim 16 where J is phenyl, optionally substituted with one or more halogen substituents.

20. A method as claimed in claim 19 where J is phenyl, *p*-chlorophenyl, *p*-fluorophenyl, or *m*-chlorophenyl.

21. A method as claimed in claim 16 where J is heteroaryl, 4-pyridyl, aralkyl, benzylthio, or $-CH_2CH_2(NH_2)COOH$.

22. A method as claimed in claim 1 where the compound of the formula (I) is a compound of the formula (V):



(V)

where T is aryl, aralkyl or alkyl, each of which is optionally substituted with one or more substituents selected from hydroxy, halogen, methoxy, amino, carboxy, and straight- or branched-chain C_1-C_6 alkyl;
or a pharmaceutically acceptable salt thereof, or a prodrug thereof.

23. A method as claimed in claim 22 where T is C_1-C_6 alkyl, optionally substituted with one or more substituents selected from halogen and hydroxy.

24. A method as claimed in claim 23 where T is methyl, ethyl, 2-fluoroethyl, or 2-hydroxyethyl.

25. A method as claimed in claim 22 where T is aryl, optionally substituted with one or more substituents selected from halogen and straight-chain C_1-C_6 alkyl.

26. A method as claimed in claim 22 where T is phenyl, naphthyl, *p*-tolyl, *m*-tolyl, *p*-chlorophenyl, *m*-chlorophenyl or *p*-fluorophenyl.

27. A method as claimed in claim 22 where T is aralkyl.

28. A method as claimed in claim 27 where T is benzyl.

29. A method as claimed in claim 1 where the compound of formula (I) is:

(3R,4R)-1-[(8-aza-9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(hydroxymethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(2-phenylethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(benzylthiomethyl)pyrrolidine;
(3R,4S)-1-[(8-aza-9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(benzylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(4-chlorophenylthiomethyl)pyrrolidine;
(3R,4R)-1-[(9-deazaadenin-9-yl)methyl]-3-acetoxy-4-(acetoxymethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(*n*-butylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(4-fluorophenylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(*n*-propylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclohexylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(3-chlorophenylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(ethylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(phenylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(4-pyridylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-*n*-propylpyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(homocysteinylmethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(benzyloxymethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(*i*-propylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(methoxymethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclohexylmethylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cycloheptylthiomethyl)pyrrolidine;
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclopentylthiomethyl)pyrrolidine; or
(3R,4S)-1-[(9-deazaadenin-9-yl)methyl]-3-hydroxy-4-(cyclobutylthiomethyl)pyrrolidine.

30. A method as claimed in any one of claims 1 to 29 where the cancer is prostate cancer or head and neck cancer.

31. A method as claimed in claim 30 where the cancer is prostate cancer.

32. A method as claimed in claim 30 where the cancer is head and neck cancer.

Figure 1A

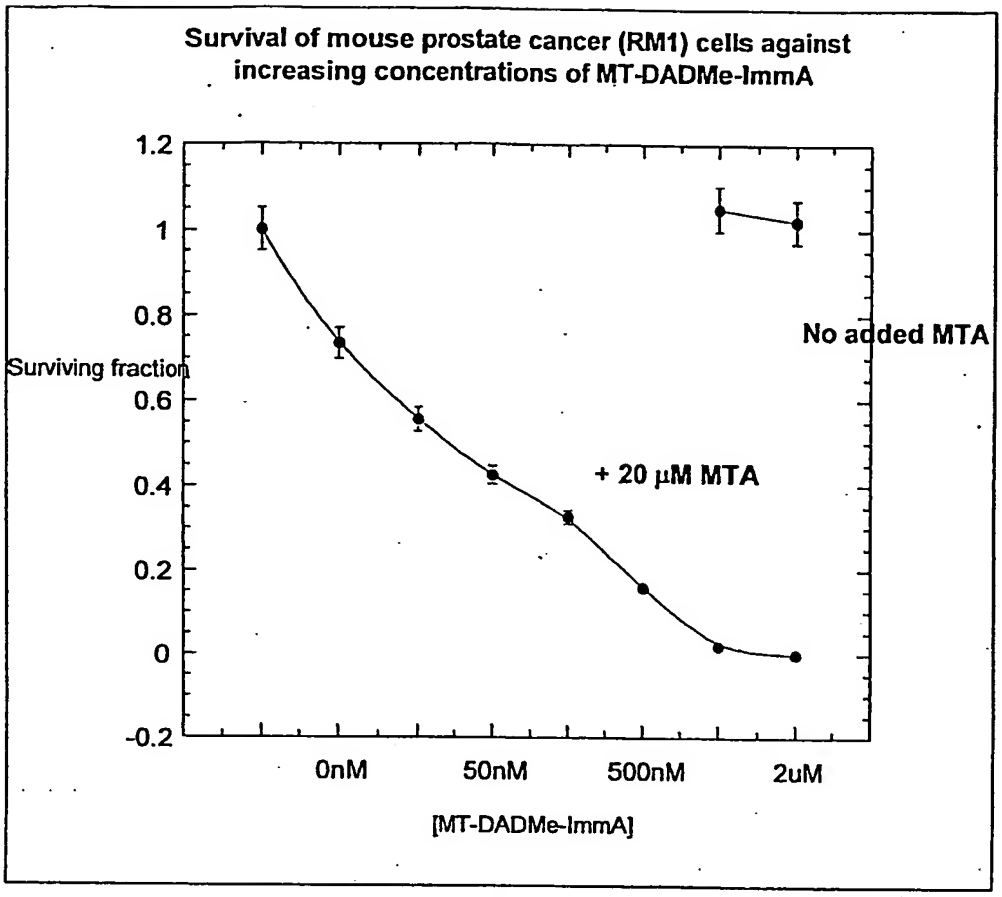


Figure 1B

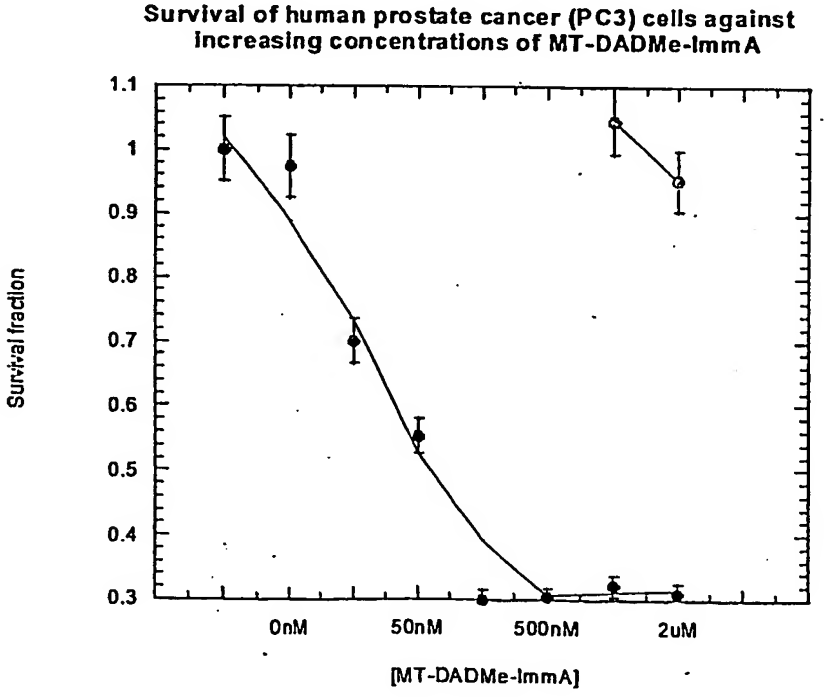


Figure 2

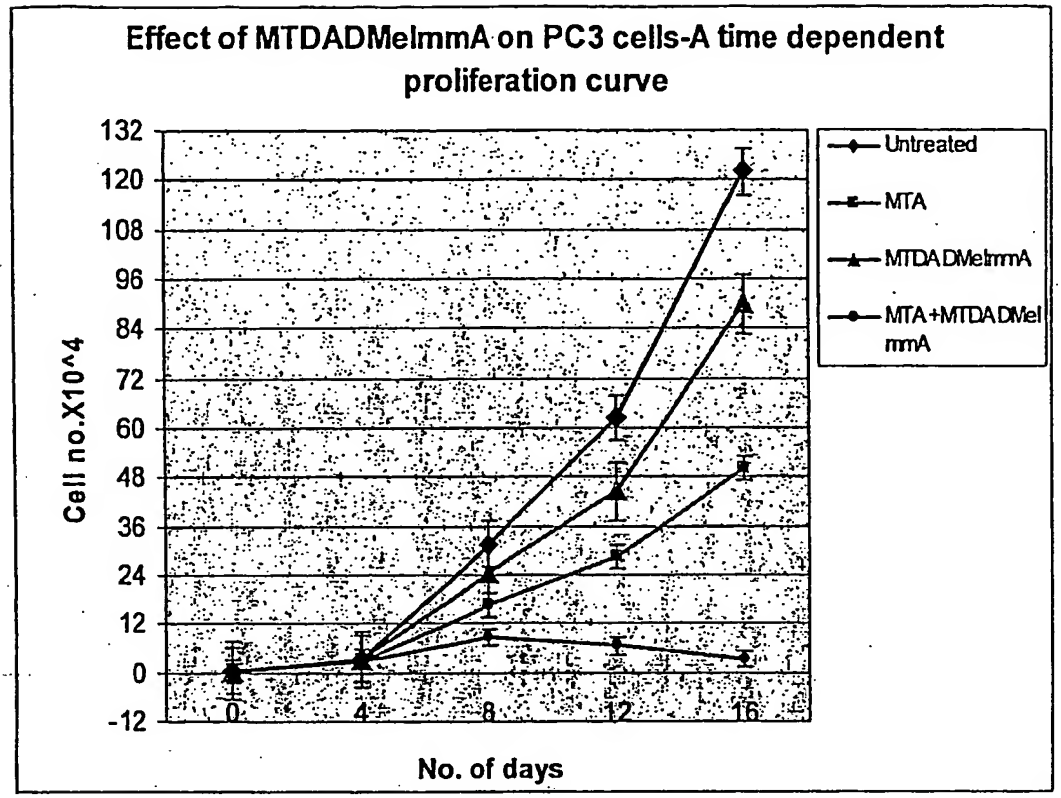

Effect of MTDADMeImmA on PC3 cells-A time dependent proliferation curve
Cell no.X10^4
132
120
108
96
84
72
60
48
36
24
12
0
-12
0
4
8
12
16
No. of days
Untreated
MTA
MTDADMeImmA
MTA+MTDADMeImmA

Figure 3

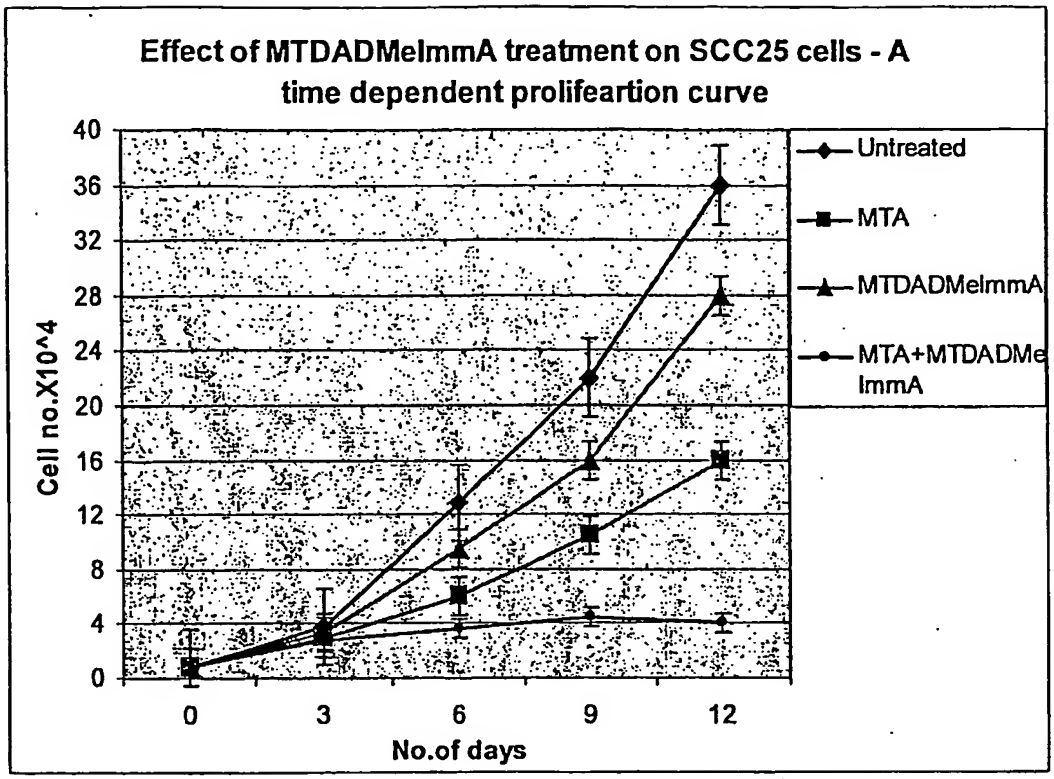

Effect of MTDADMeImmA treatment on SCC25 cells - A time dependent prolifeartion curve
Cell no.X10^4
40
36
32
28
24
20
16
12
8
4
0
0
3
6
9
12
No.of days
Untreated
MTA
MTDADMeImmA
MTA+MTDADMeImmA

Figure 4

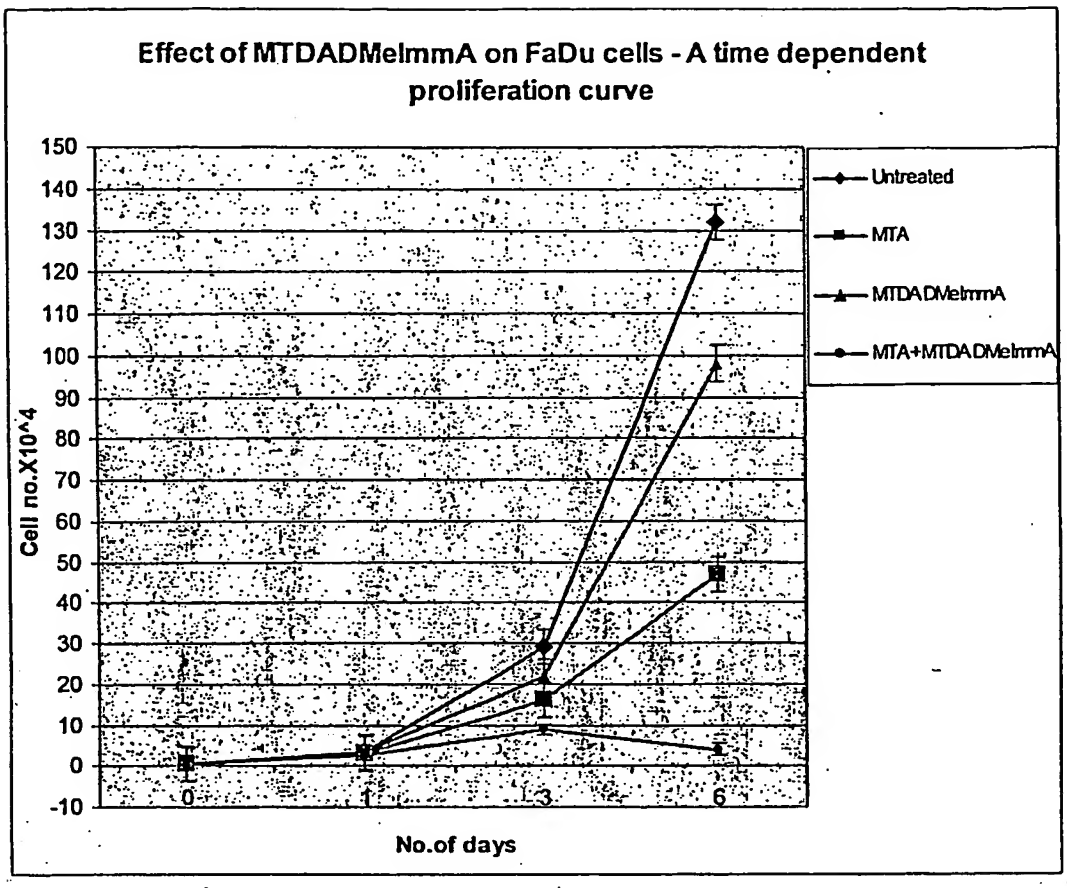

Effect of MTDADMeImmA on FaDu cells - A time dependent proliferation curve
Cell no.X10^4
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
0
1
3
6
No.of days
Untreated
MTA
MTDADMeImmA
MTA+MTDADMeImmA

Figure 5

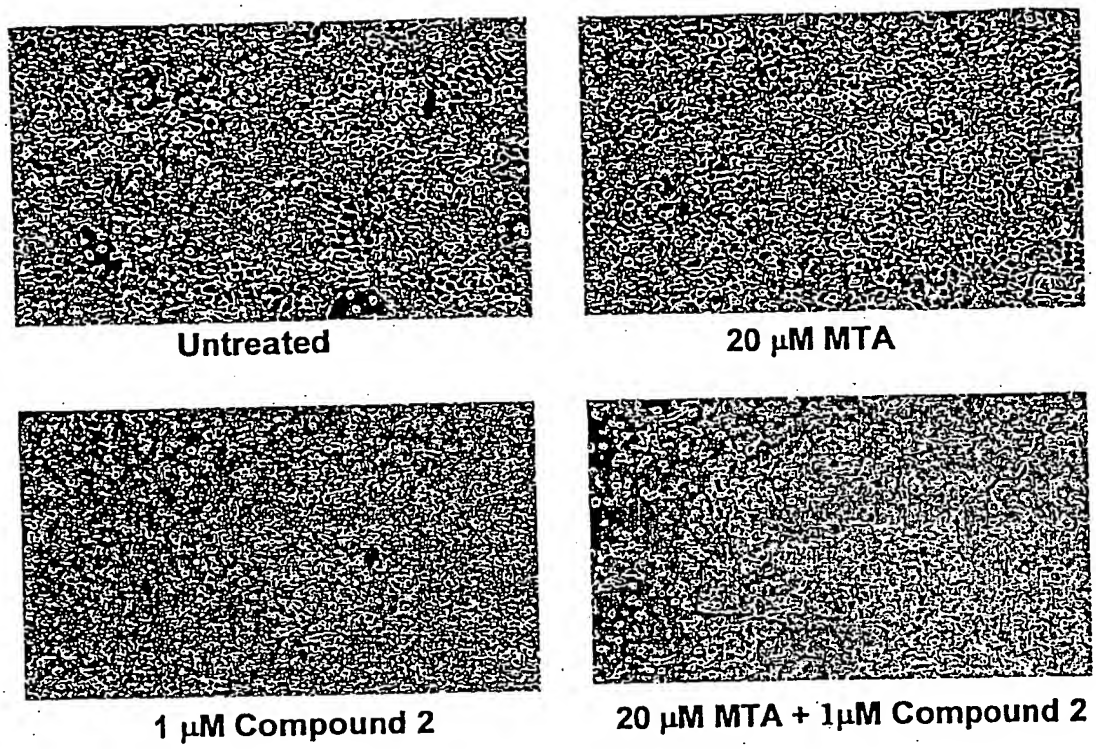


Figure 6

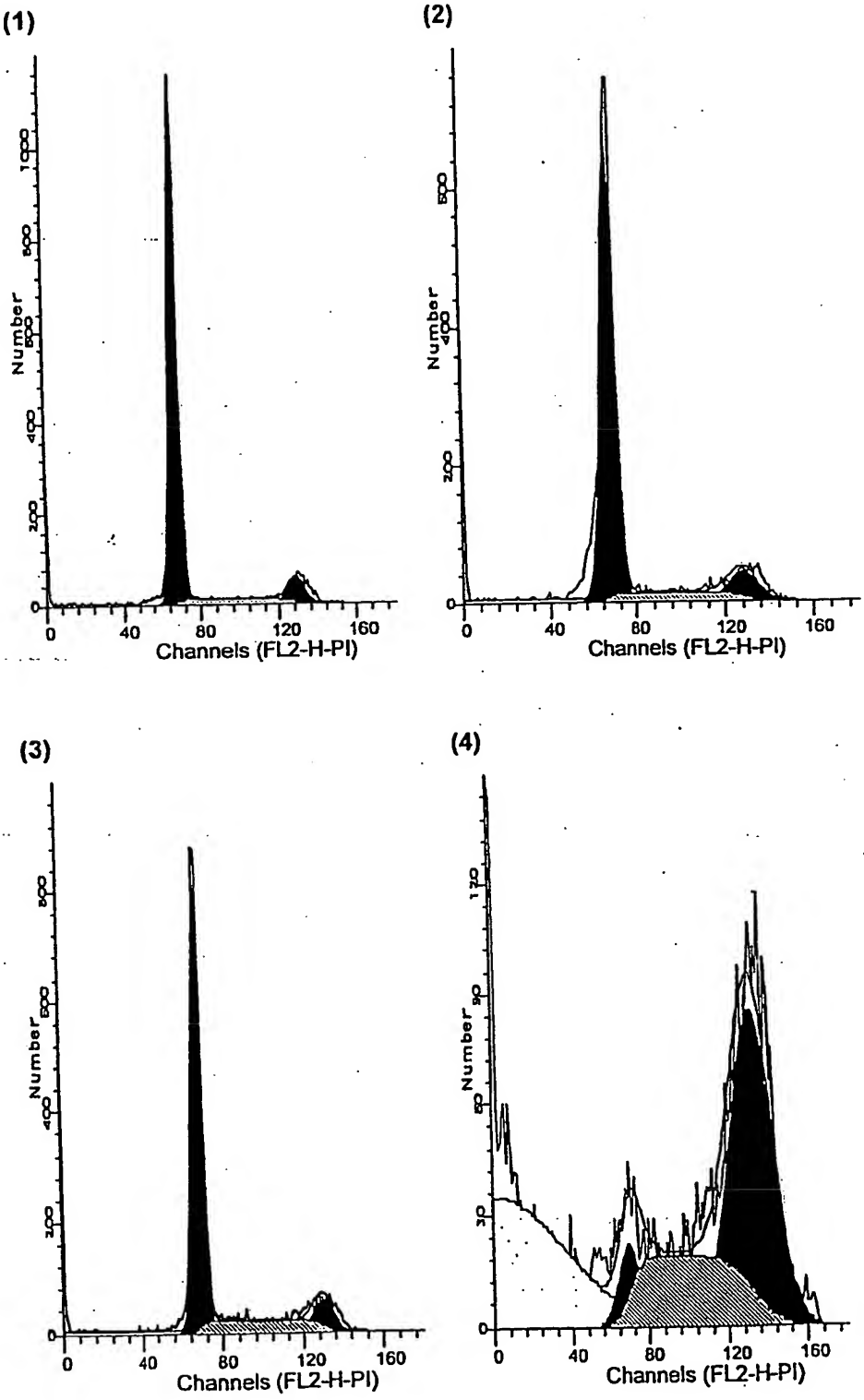

(1)
Number
Channels (FL2-H-PI)
(2)
Number
Channels (FL2-H-PI)
(3)
Number
Channels (FL2-H-PI)
(4)
Number
Channels (FL2-H-PI)

Figure 7

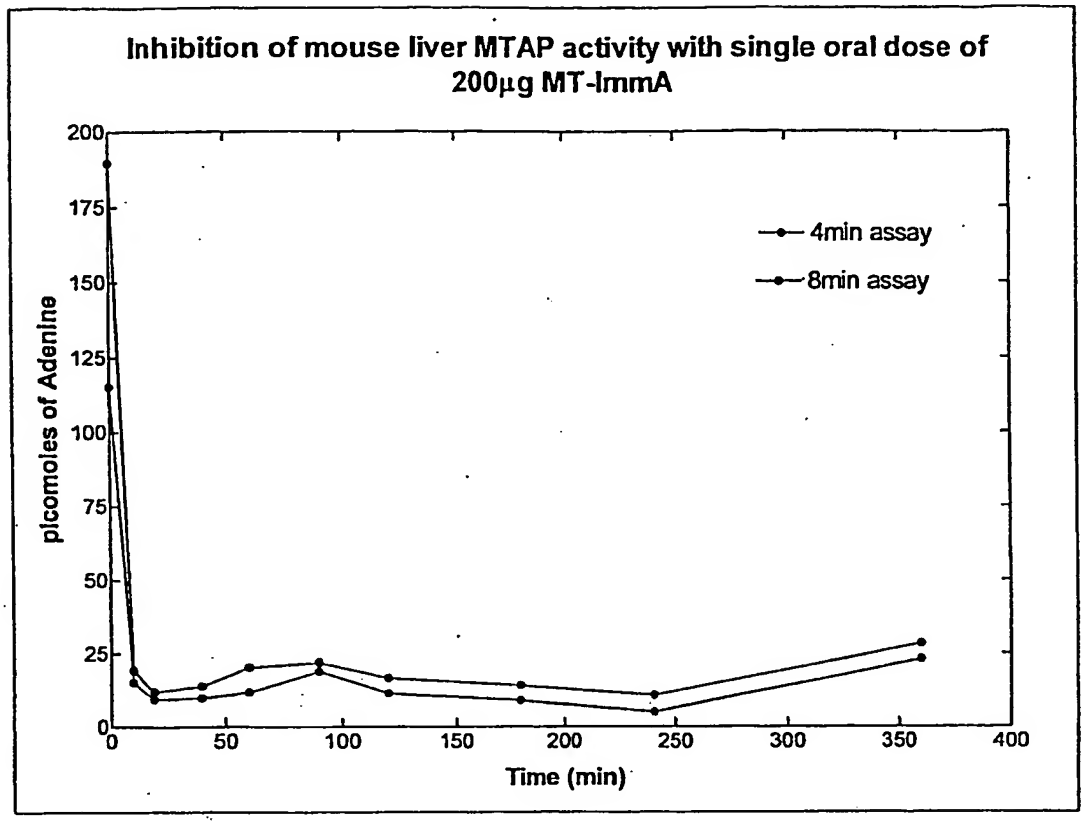

Inhibition of mouse liver MTAP activity with single oral dose of 200µg MT-ImmA
picomoles of Adenine
Time (min)
4min assay
8min assay
0
25
50
75
100
125
150
175
200
0
50
100
150
200
250
300
350
400

Figure 8

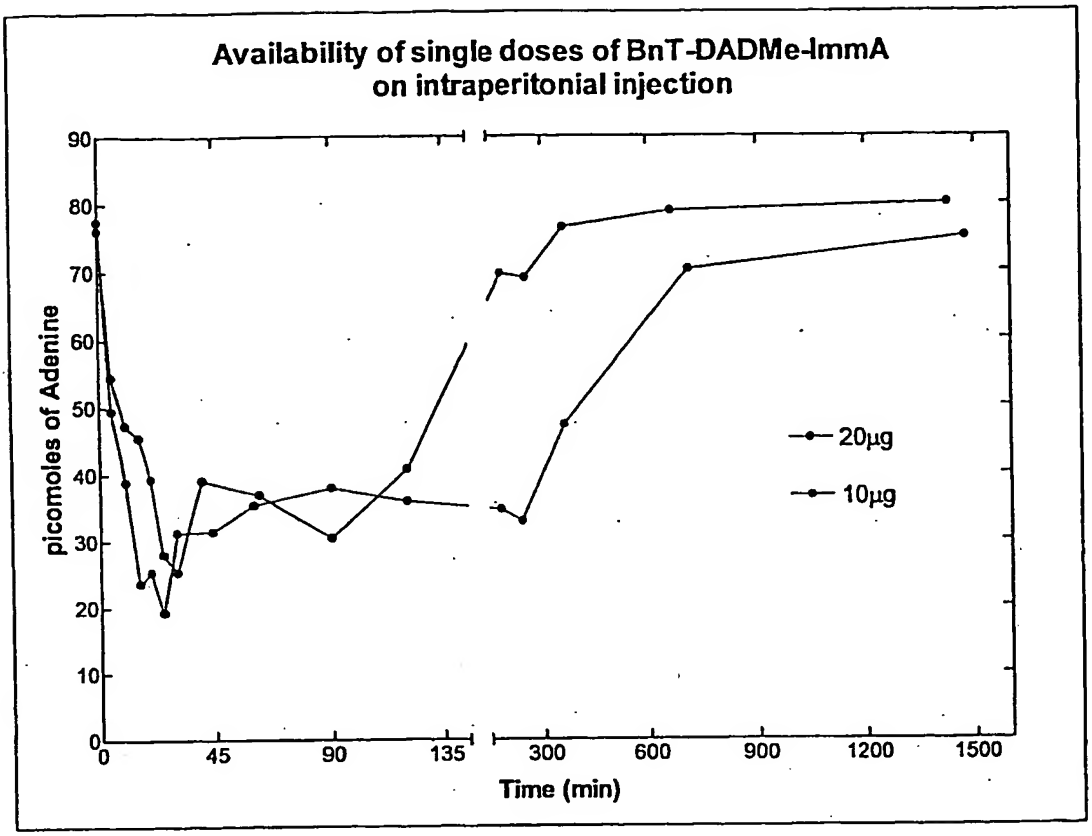

Availability of single doses of BnT-DADMe-ImmA
on intraperitonial injection
picomoles of Adenine
Time (min)
20µg
10µg
0
10
20
30
40
50
60
70
80
90
0
45
90
135
300
600
900
1200
1500

Figure 9

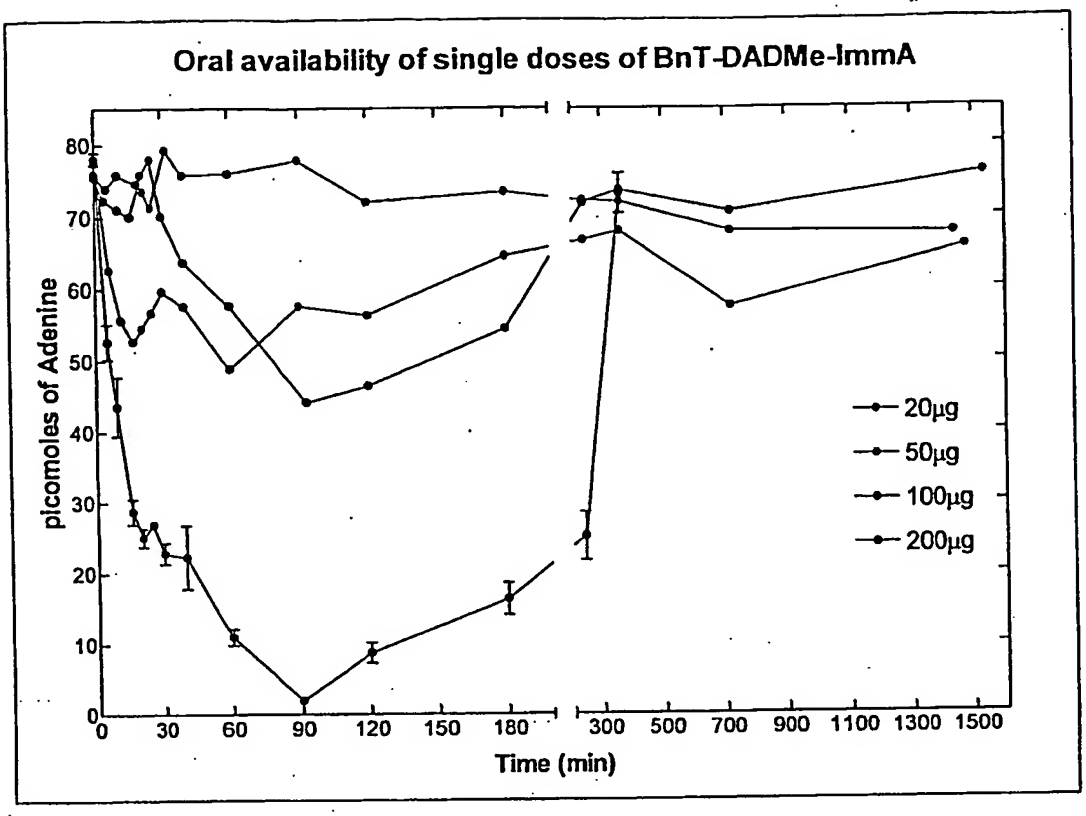

Oral availability of single doses of BnT-DADMe-ImmA
picomoles of Adenine
Time (min)
20µg
50µg
100µg
200µg
0
10
20
30
40
50
60
70
80
0
30
60
90
120
150
180
300
500
700
900
1100
1300
1500

Figure 10

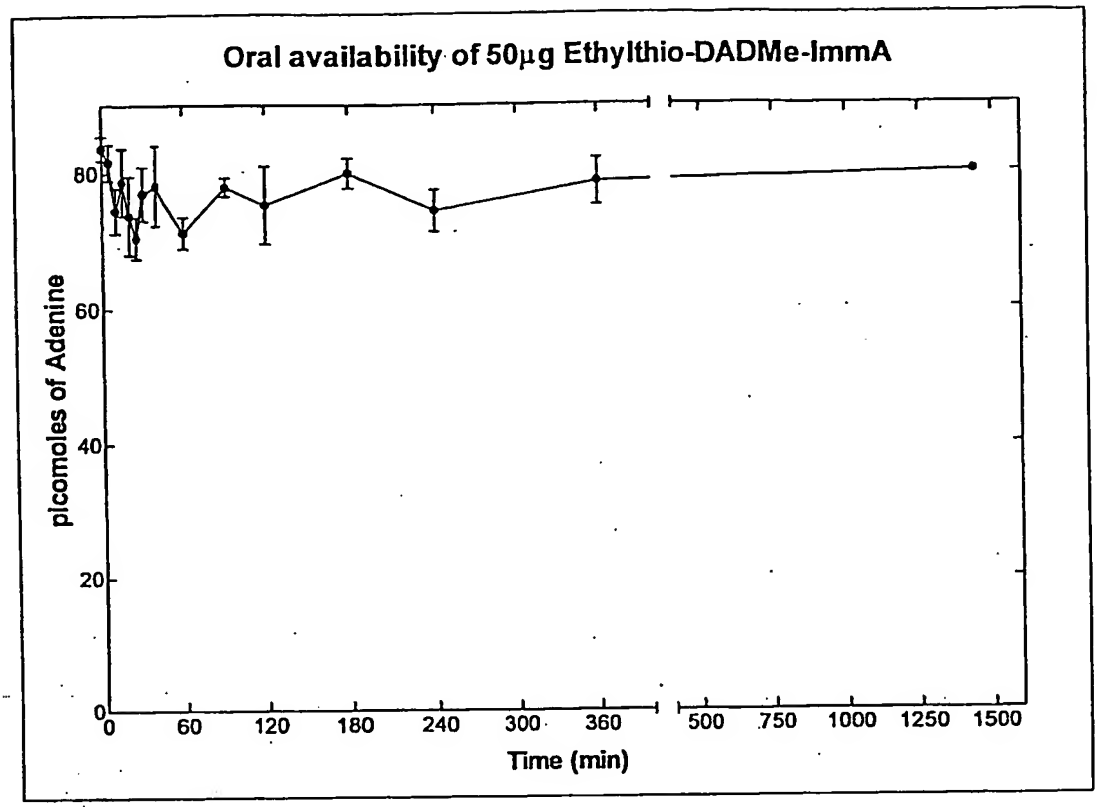

Oral availability of 50µg Ethylthio-DADMe-ImmA
picomoles of Adenine
0
20
40
60
80
0
60
120
180
240
300
360
500
750
1000
1250
1500
Time (min)

Figure 11

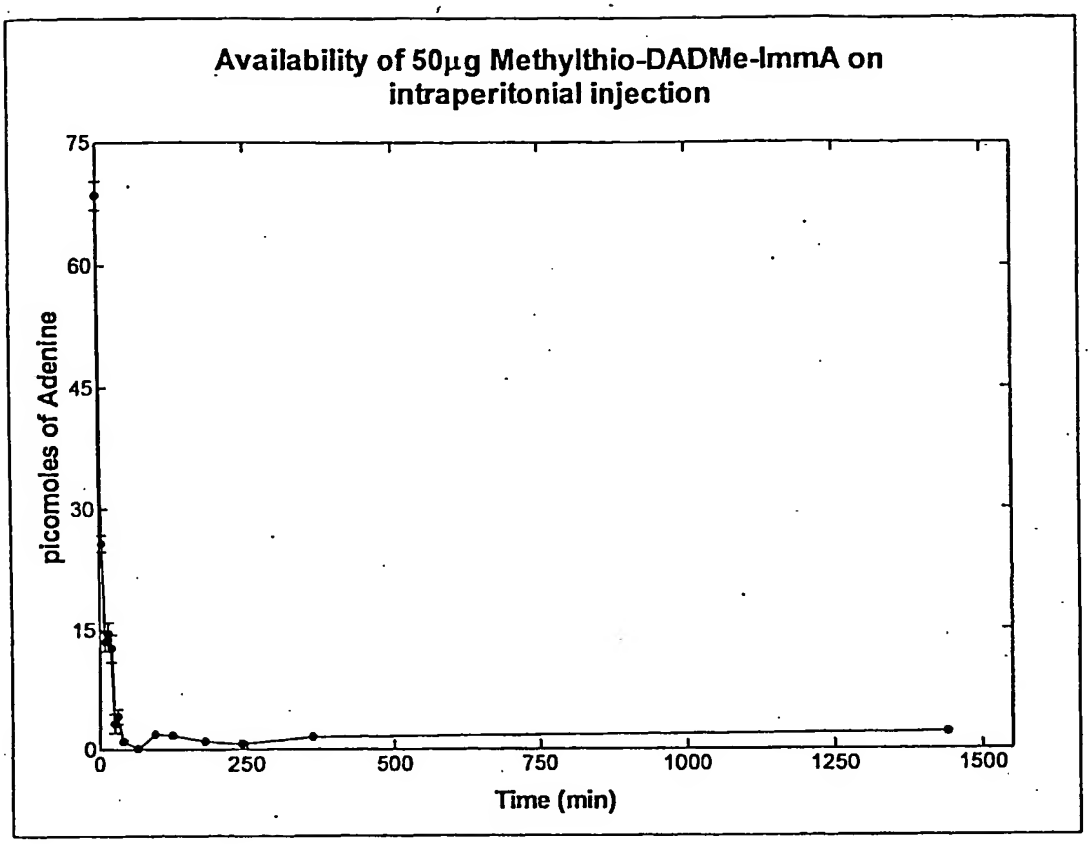

Availability of 50µg Methylthio-DADMe-ImmA on intraperitonial injection
picomoles of Adenine
75
60
45
30
15
0
0
250
500
750
1000
1250
1500
Time (min)

Figure 12

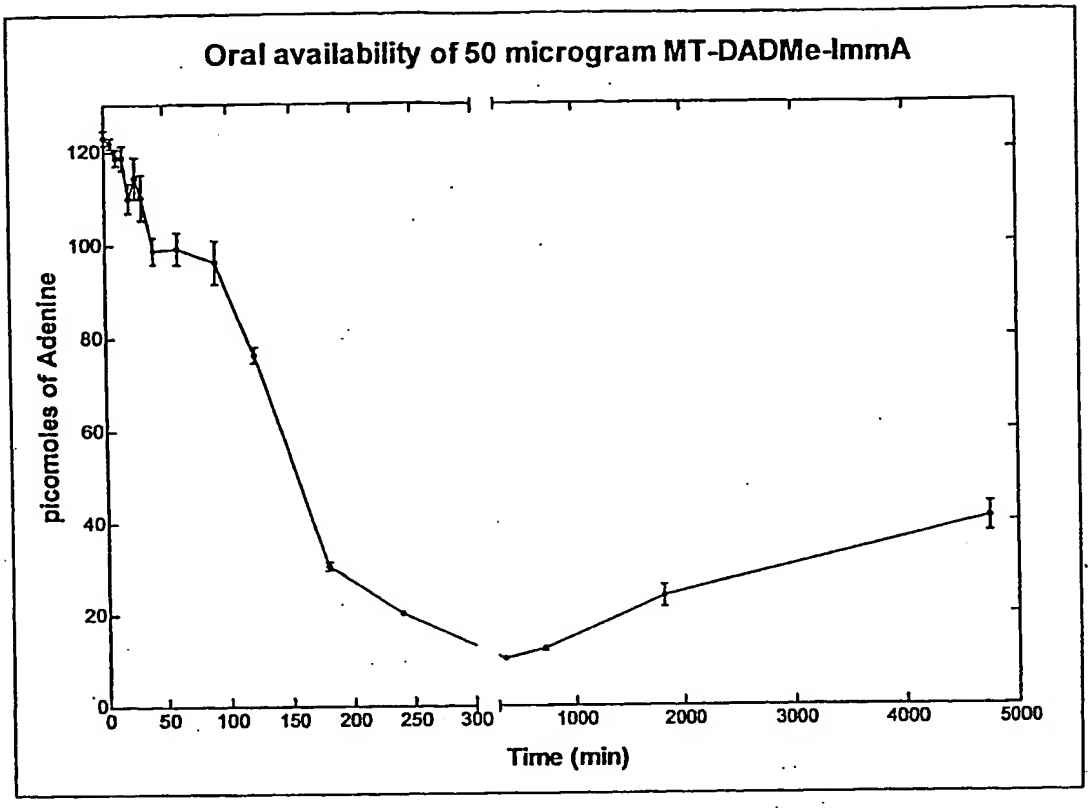

Oral availability of 50 microgram MT-DADMe-ImmA
picomoles of Adenine
120
100
80
60
40
20
0
0
50
100
150
200
250
300
1000
2000
3000
4000
5000
Time (min)

Figure 13

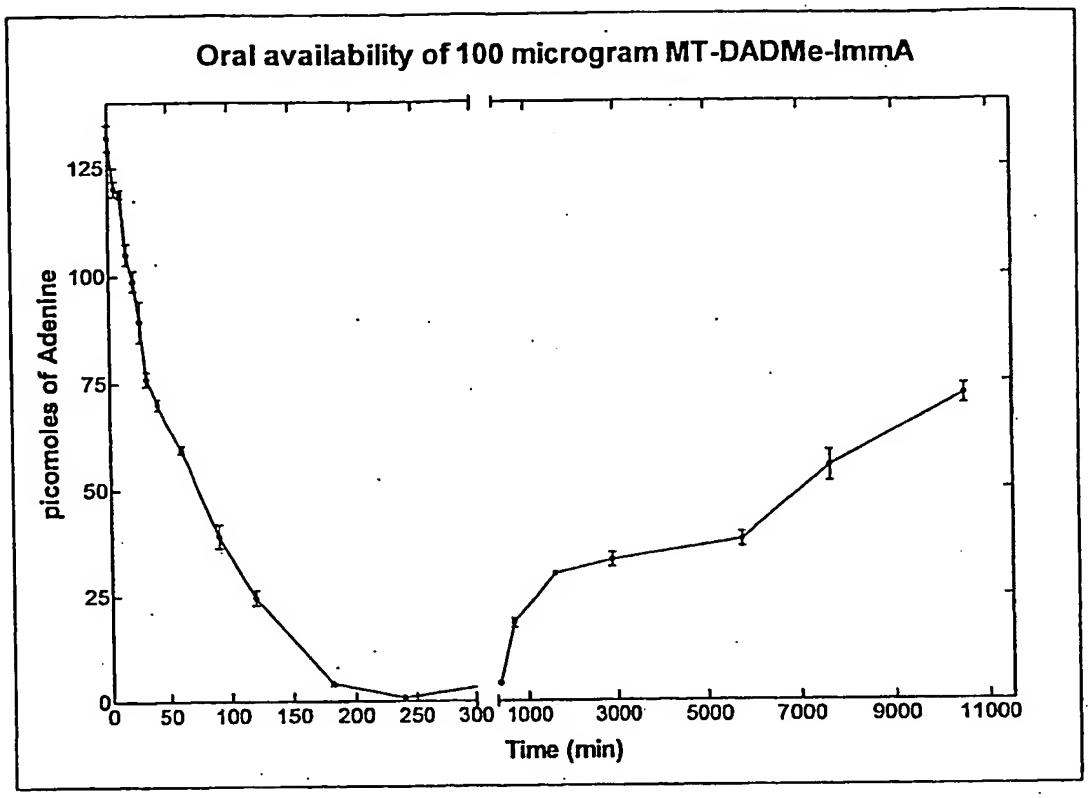

Oral availability of 100 microgram MT-DADMe-ImmA
picomoles of Adenine
125
100
75
50
25
0
0
50
100
150
200
250
300
1000
3000
5000
7000
9000
11000
Time (min)

Figure 14

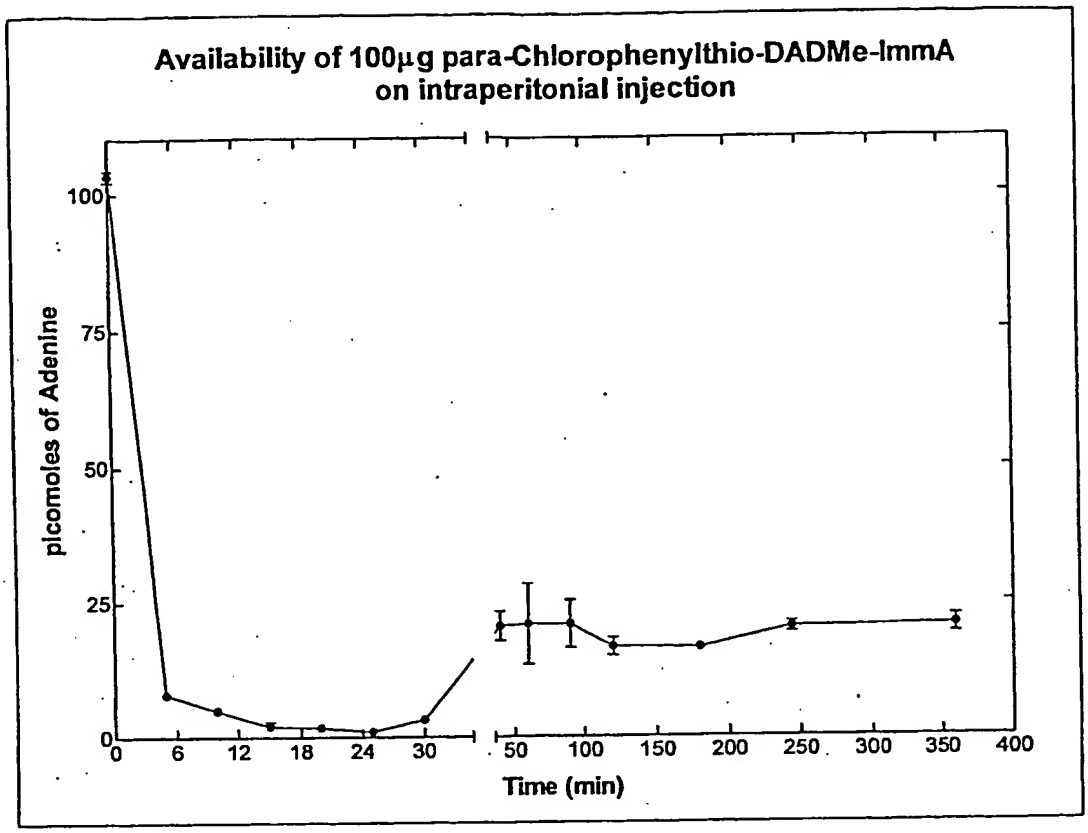

Availability of 100µg para-Chlorophenylthio-DADMe-ImmA
on intraperitonial injection
picomoles of Adenine
100
75
50
25
0
0
6
12
18
24
30
50
100
150
200
250
300
350
400
Time (min)

Figure 15

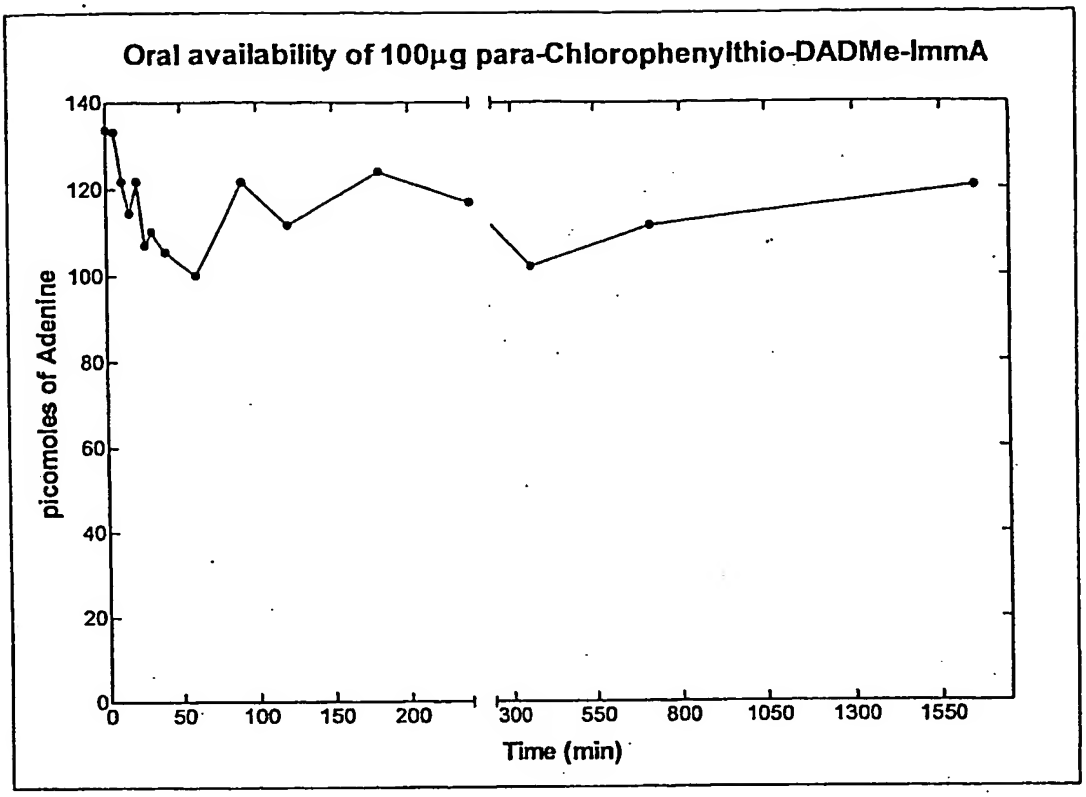

Oral availability of 100µg para-Chlorophenylthio-DADMe-ImmA
picomoles of Adenine
140
120
100
80
60
40
20
0
0
50
100
150
200
300
550
800
1050
1300
1550
Time (min)

Figure 16

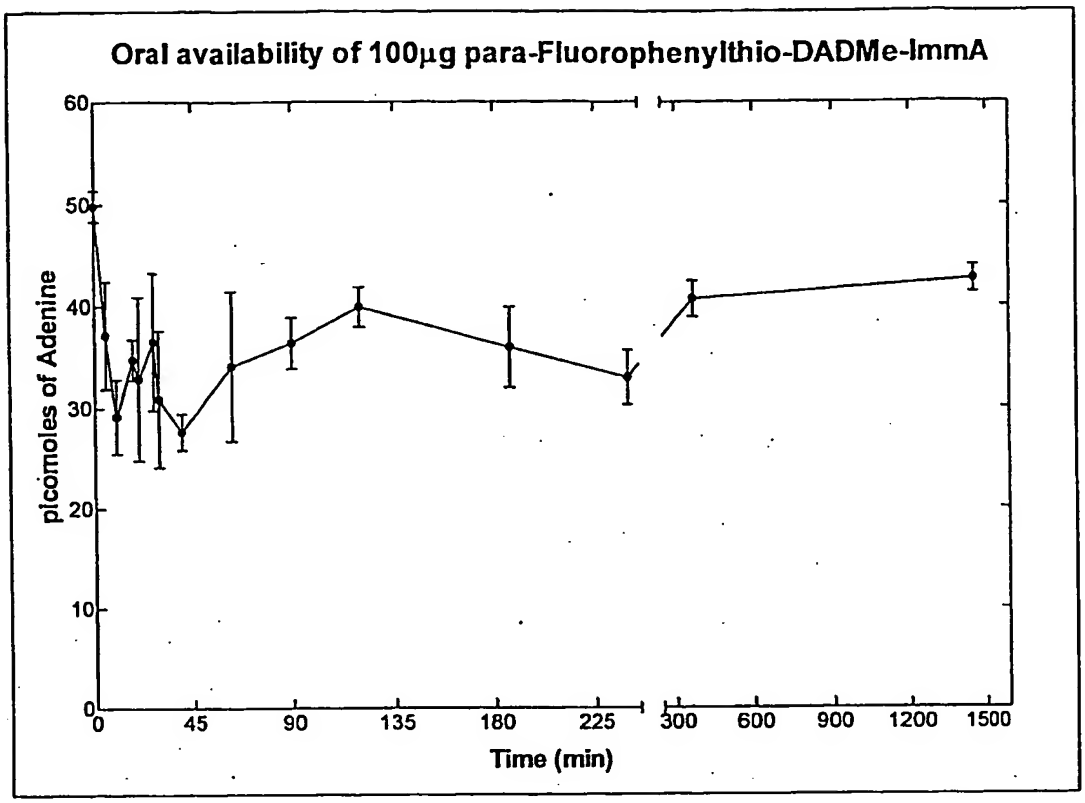

Oral availability of 100µg para-Fluorophenylthio-DADMe-ImmA
picomoles of Adenine
60
50
40
30
20
10
0
0
45
90
135
180
225
300
600
900
1200
1500
Time (min)

Figure 17

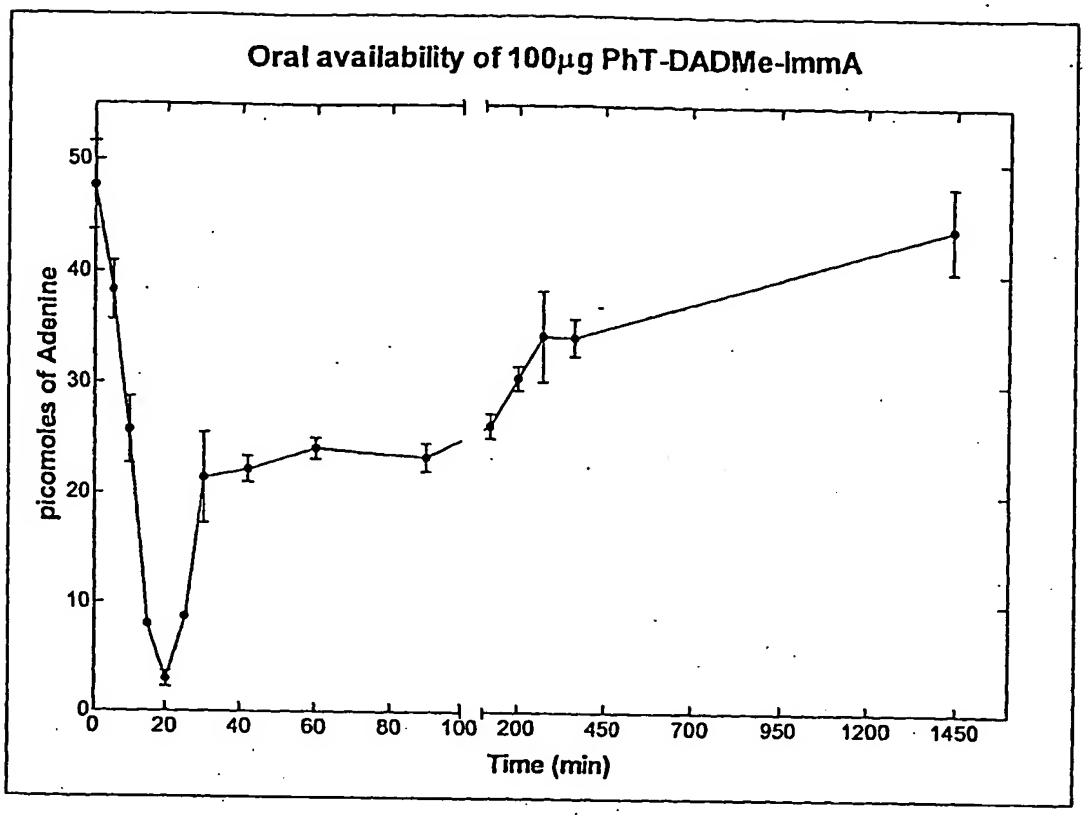

Oral availability of 100μg PhT-DADMe-ImmA
picomoles of Adenine
0
10
20
30
40
50
0
20
40
60
80
100
200
450
700
950
1200
1450
Time (min)

Figure 18

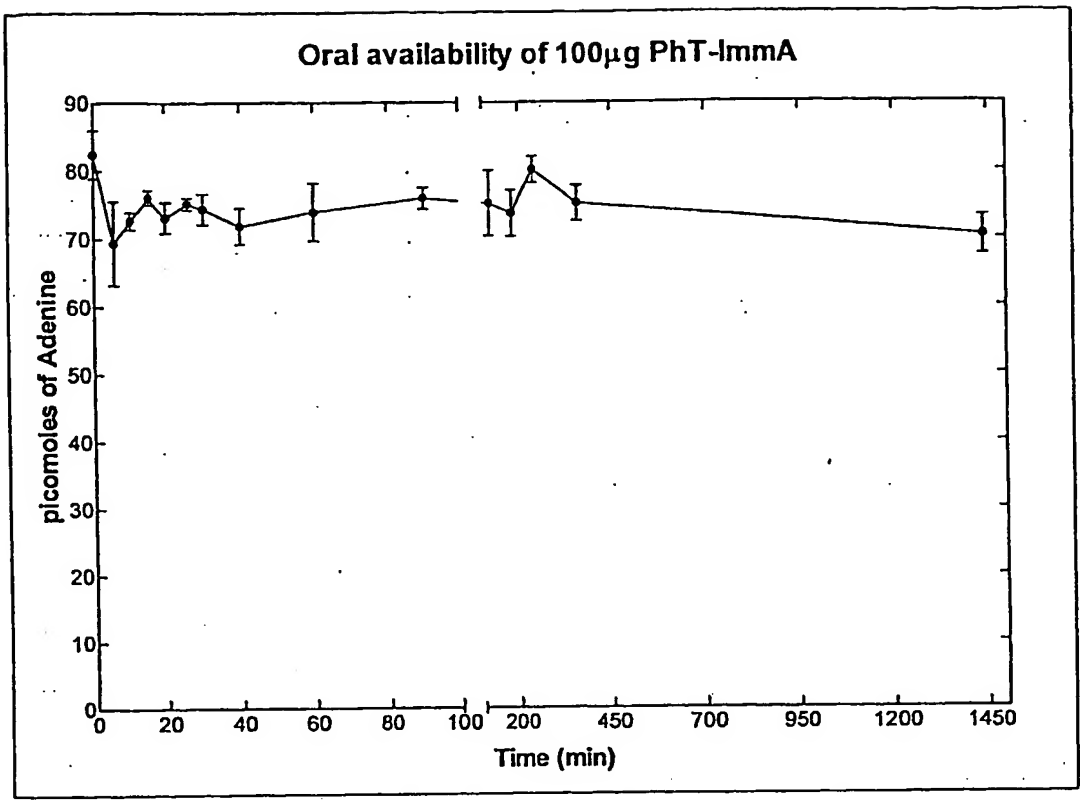

Oral availability of 100µg PhT-ImmA
picomoles of Adenine
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
200
450
700
950
1200
1450
Time (min)

Figure 19

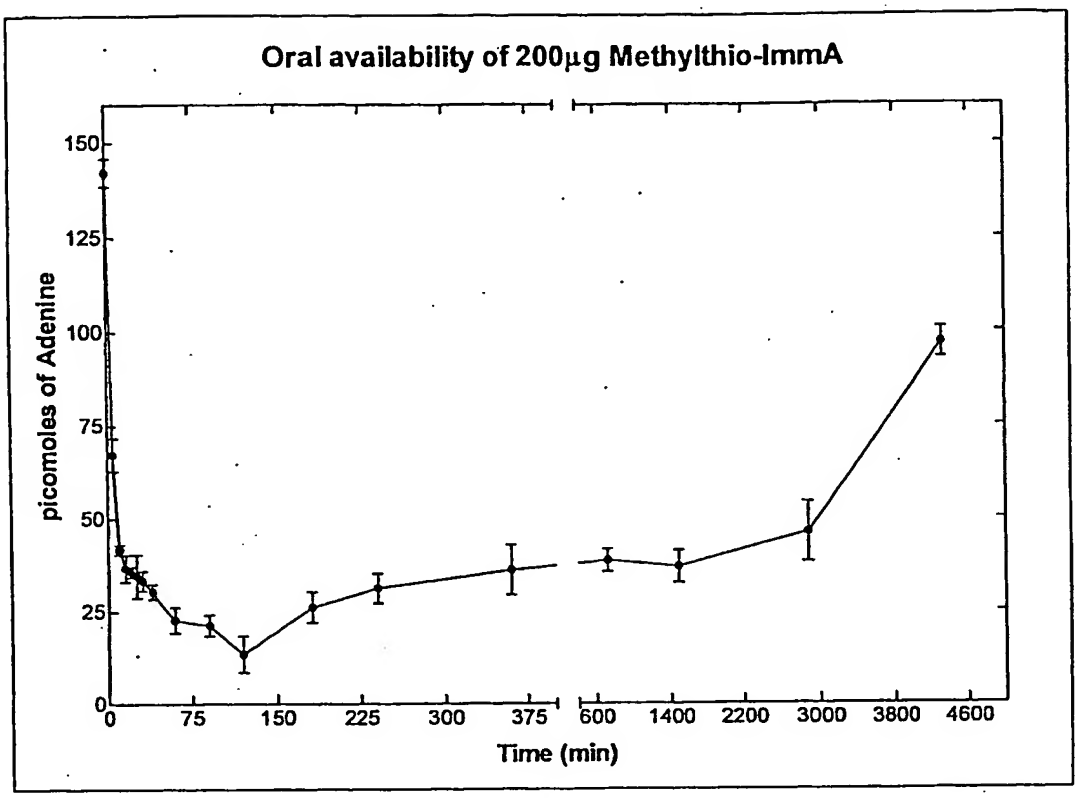

Oral availability of 200µg Methylthio-ImmA
picomoles of Adenine
150
125
100
75
50
25
0
0
75
150
225
300
375
600
1400
2200
3000
3800
4600
Time (min)

Figure 20

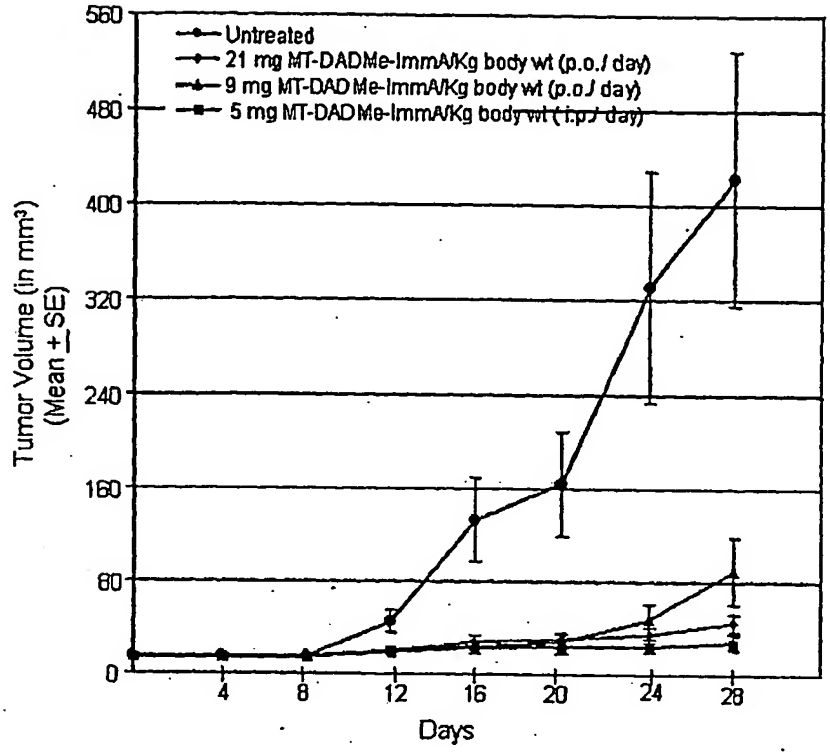

Untreated
21 mg MT-DADMe-ImmA/Kg body wt (p.o./ day)
9 mg MT-DADMe-ImmA/Kg body wt (p.o./ day)
5 mg MT-DADMe-ImmA/Kg body wt (i.p./ day)
Tumor Volume (in mm³)
(Mean +_SE)
560
480
400
320
240
160
80
0
4
8
12
16
20
24
28
Days

Figure 21

Untreated | 21 mg/Kg body wt. (p.o./day) | 9 mg/Kg body wt. (p.o./day) | 5 mg/Kg body wt. (i.p./day)

Figure 22

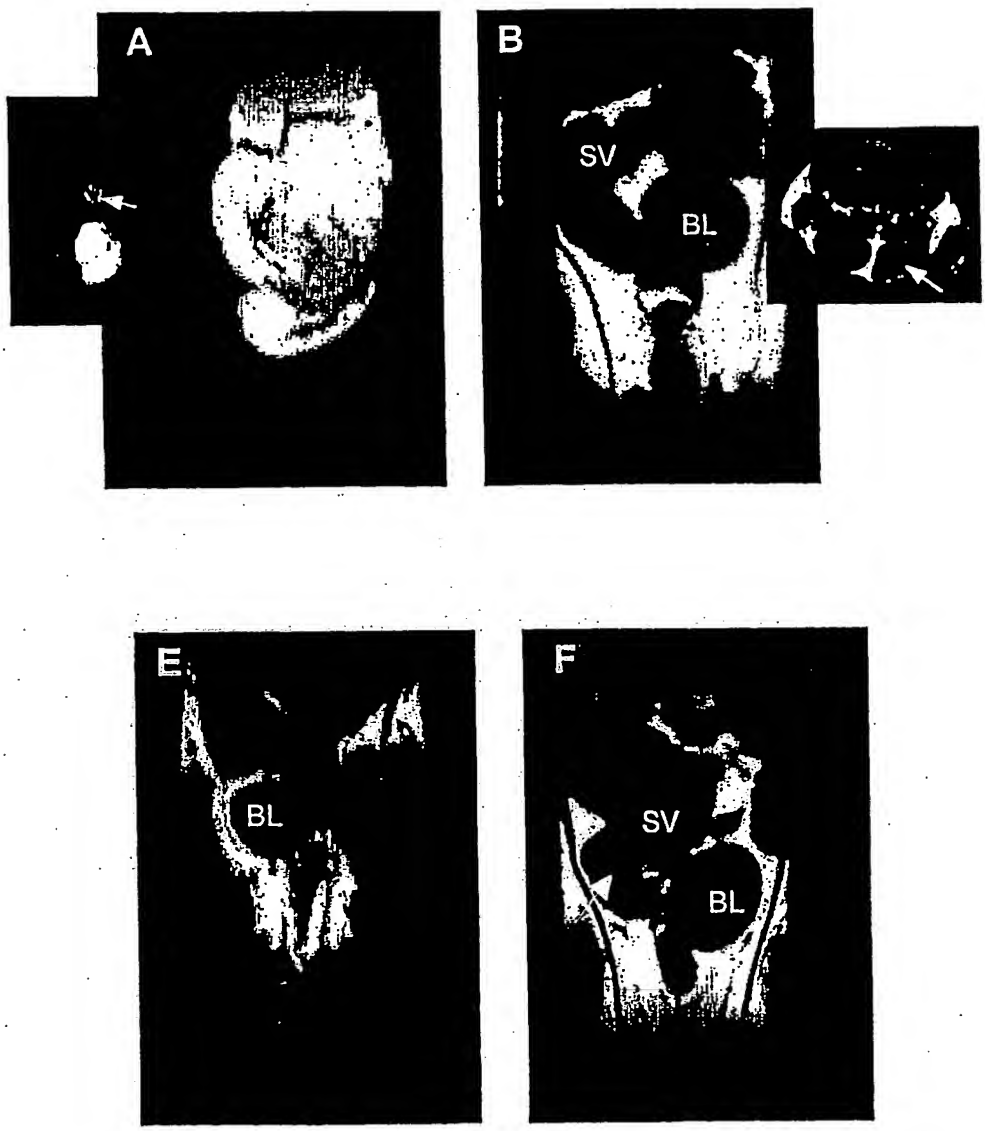
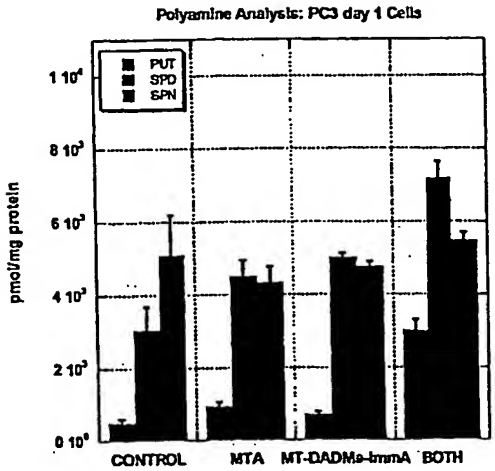
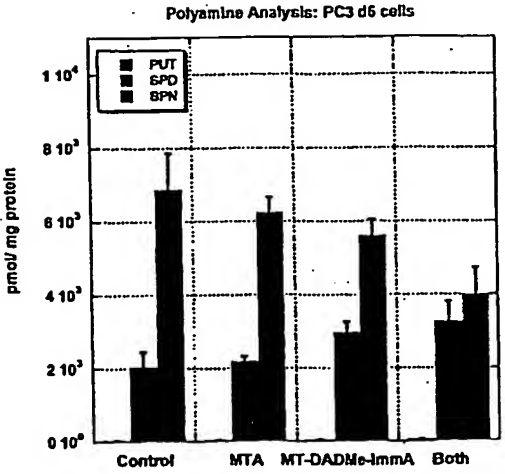
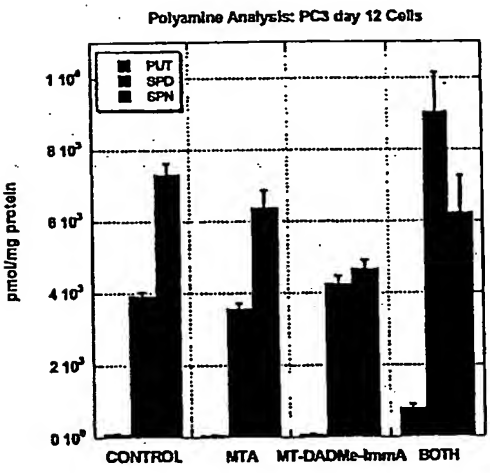
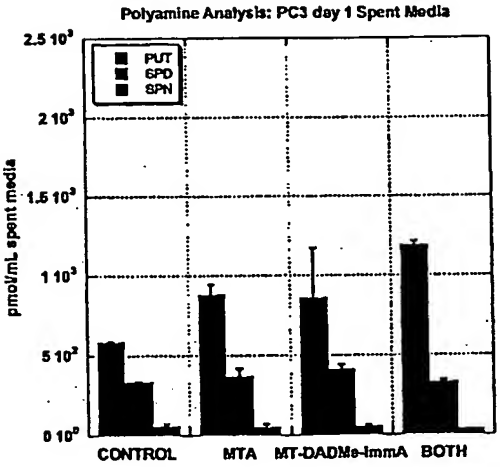


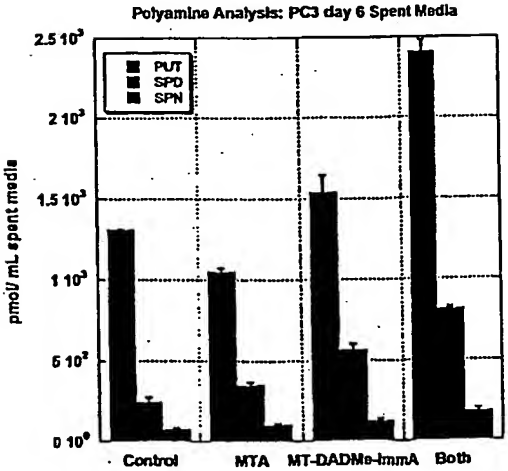
Figure 23


Polyamine Analysis: PC3 day 1 Cells
PUT
SPD
SPN
pmol/mg protein
1 10^4
8 10^3
6 10^3
4 10^3
2 10^3
0 10^0
CONTROL
MTA
MT-DADMe-ImmA
BOTH


Polyamine Analysis: PC3 d6 cells
PUT
SPD
SPN
pmol/ mg protein
1 10^4
8 10^3
6 10^3
4 10^3
2 10^3
0 10^0
Control
MTA
MT-DADMe-ImmA
Both


Polyamine Analysis: PC3 day 12 Cells
PUT
SPD
SPN
pmol/mg protein
1 10^4
8 10^3
6 10^3
4 10^3
2 10^3
0 10^0
CONTROL
MTA
MT-DADMe-ImmA
BOTH


Polyamine Analysis: PC3 day 1 Spent Media
PUT
SPD
SPN
pmol/mL spent media
2.5 10^3
2 10^3
1.5 10^3
1 10^3
5 10^2
0 10^0
CONTROL
MTA
MT-DADMe-ImmA
BOTH


Polyamine Analysis: PC3 day 6 Spent Media
PUT
SPD
SPN
pmol/ mL spent media
2.5 10^3
2 10^3
1.5 10^3
1 10^3
5 10^2
0 10^0
Control
MTA
MT-DADMe-ImmA
Both

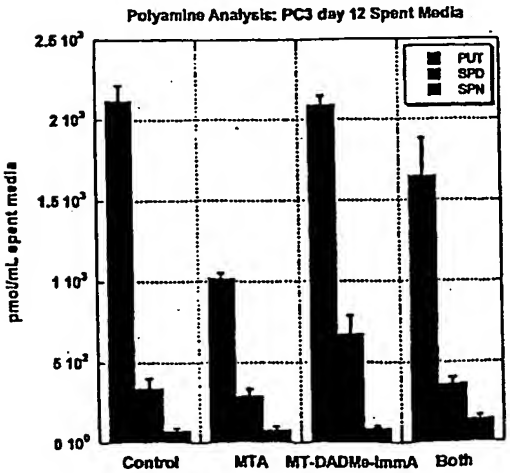

Polyamine Analysis: PC3 day 12 Spent Media
PUT
SPD
SPN
pmol/mL spent media
2.5 10^3
2 10^3
1.5 10^3
1 10^3
5 10^2
0 10^0
Control
MTA
MT-DADMe-ImmA
Both

Figure 24A

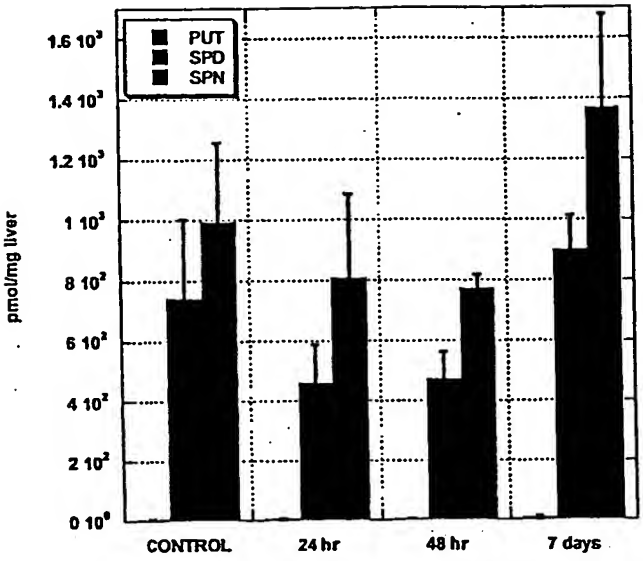


Figure 24B

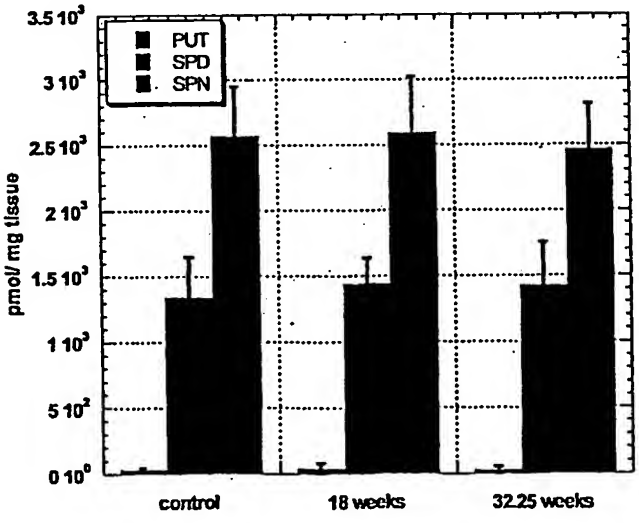


Figure 24C

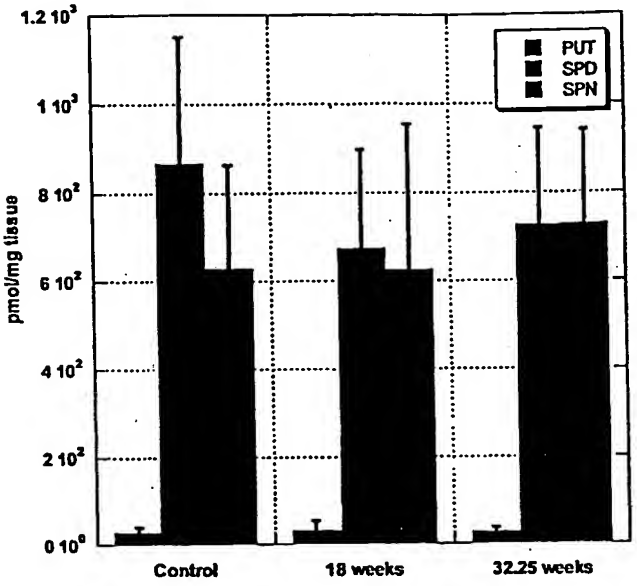

1.2 10³
1 10³
8 10²
6 10²
4 10²
2 10²
0 10⁰
pmol/mg tissue
PUT
SPD
SPN
Control
18 weeks
32.25 weeks

Figure 25A

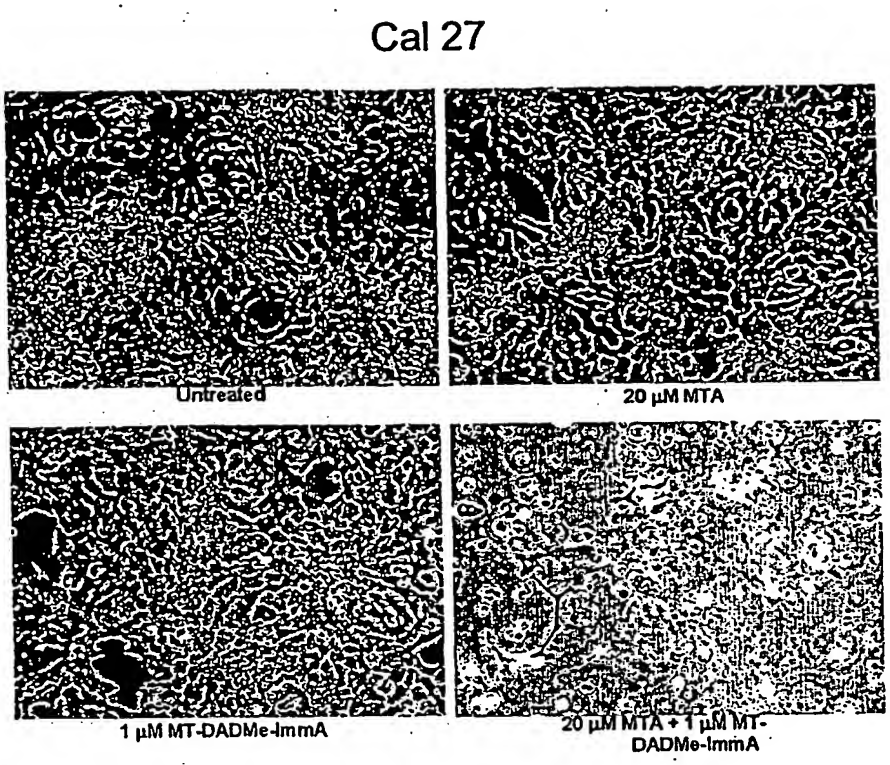


Figure 25B

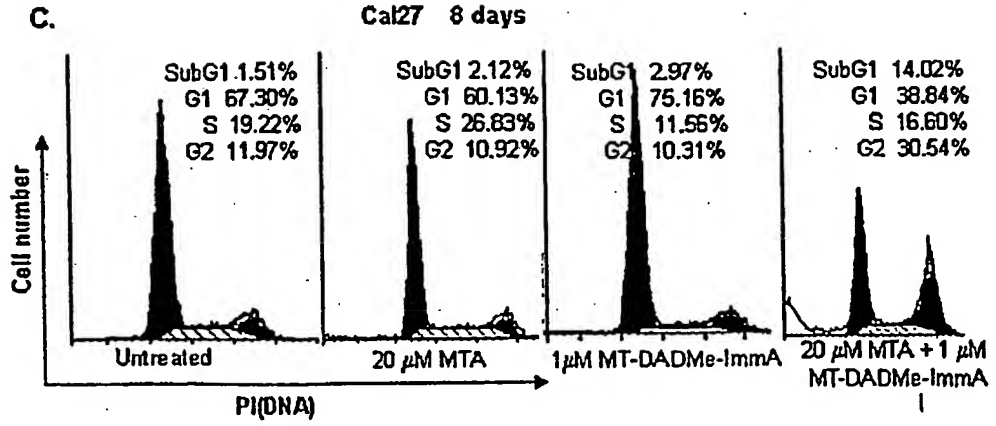
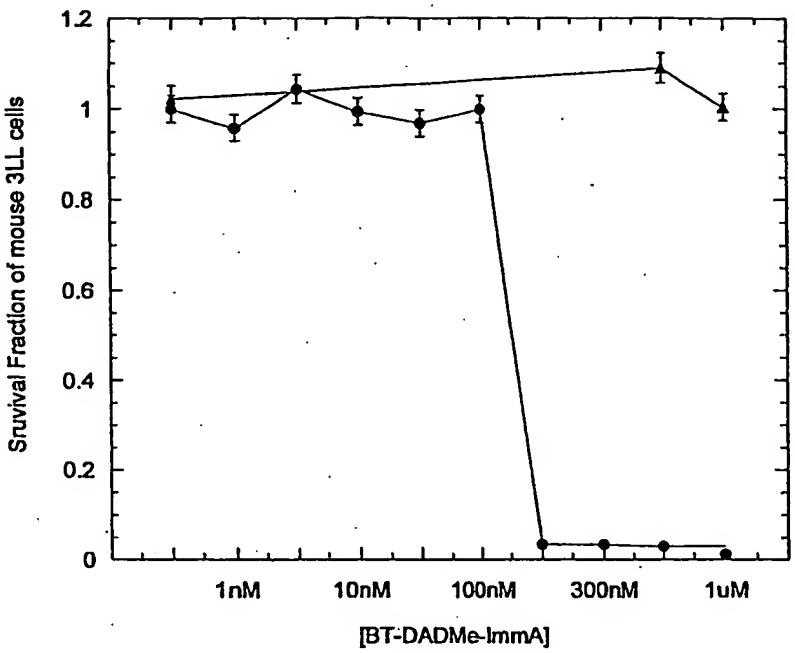


Figure 26


1.2
1
0.8
0.6
0.4
0.2
0
Sruvival Fraction of mouse 3LL cells
1nM
10nM
100nM
300nM
1uM
[BT-DADMe-ImmA]

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ2007/000036

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. *A61K 31/519* (2006.01) *A61K 31/7042* (2006.01) *A61P 35/00* (2006.01)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DWPI, CAPLUS, MEDLINE. keywords- tumor, tumour, cancer, inhibit, MTAP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/018496 A1 (Albert Einstein College of Medicine of Yeshiva University) 4 March 2004.	1-32
X	WO 2006/014913 A2 (Biocryst Pharmaceuticals, Inc) 9 February 2006	1-15, 22-32
X	WO 2003/080620 A1 (Industrial Research Limited) 2 October 2003	1-15, 22-32
X	US 6,458,799 B1 (Biocryst Pharmaceuticals, Inc) 1 October 2002	1-15, 22-32

☐ Further documents are listed in the continuation of Box C ☒ See patent family annex

* Special categories of cited documents:

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
28 May 2007

Date of mailing of the international search report
31 MAY 2007

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/NZ2007/000036

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited In Search Report		Patent Family Member					
WO	2004/018496	AU	2003258911	CA	2496698	EP	1539783
		RU	2005107714	US	2006160765		
WO	2006/014913	AU	2005269541	EP	1771452		
WO	2003/080620	AU	2003215969	CA	2480470	EP	1490373
		US	2004110772				

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX